SEA LICE 2014

The 10th International Sea Lice Conference

Portland, Maine 31st August-5th September 2014

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Sunday night reception
Thursday pm coffee break

Sponsoring session: *"Use of chemotherapeutants, modes of action, targets, and resistance I"*
Subsidized student registrations
Tuesday pm coffee break
Lobster bake

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Poster session
Lunch on Tuesday

Student oral and poster awards
Lunch on Monday

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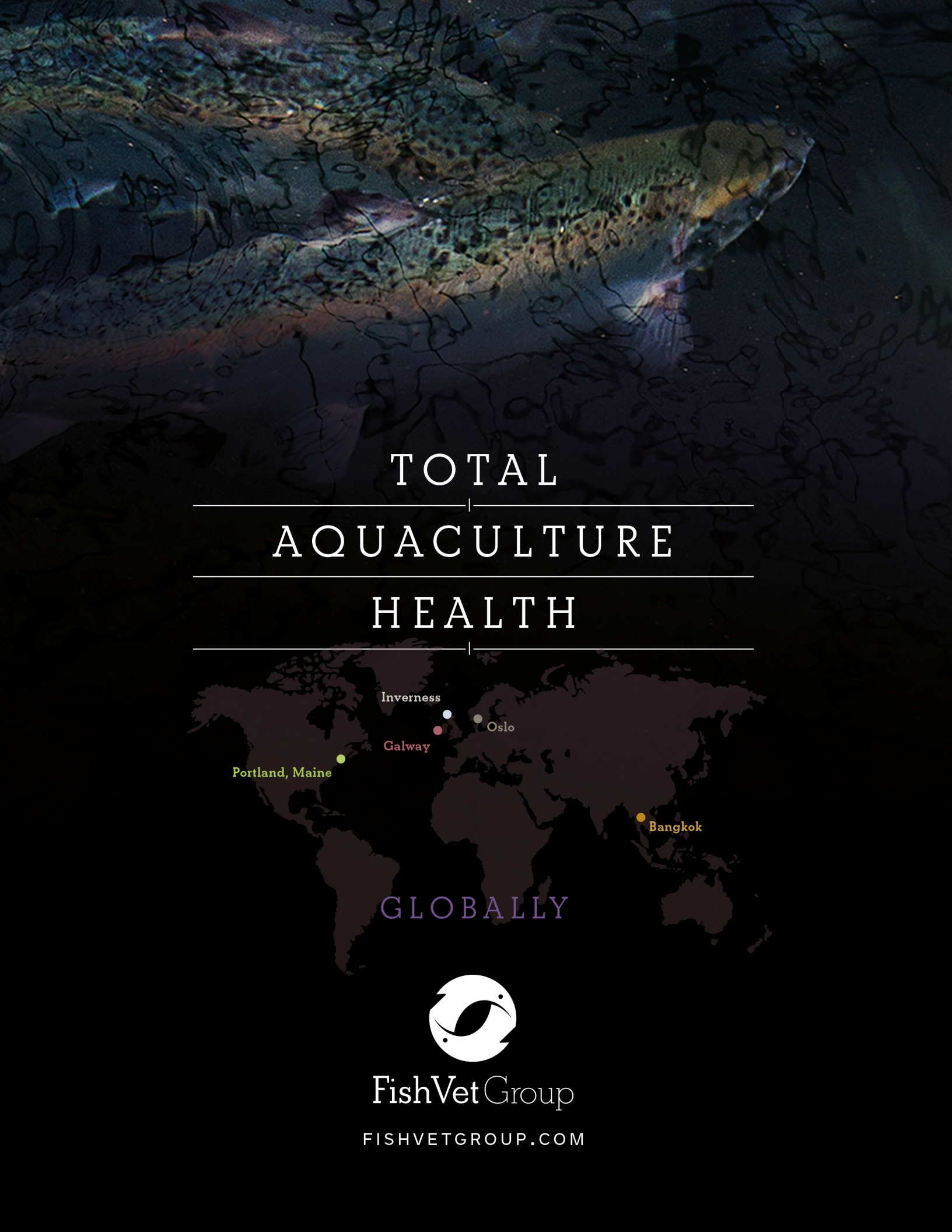
Breakfast on Friday

Coffee break on Monday am

'Young scientist award'

Coffee break on Monday pm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	Sunday	Monday	Tuesday
	August 31, 2014	September 1, 2014	September 2, 2014
7:00-8:00 am		Breakfast	Breakfast
8:00-8:15 am		Welcome	Plenary: Stephen Bocking
8:15-8:30 am			
8:30-8:45 am			
8:45-9:00 am			
9:00-9:15 am		Plenary: Nell Halse	Epidemiology, Modeling and Analysis
9:15-9:30 am			
9:30-9:45 am			
9:45-10:00 am			
10:00-10:15 am		Break	
10:15-10:30 am			
10:30-10:45 am		Coordinated Treatments and Sustainable Production	Break
10:45-11:00 am			
11:00-11:15 am			Sea Louse Biology I
11:15-11:30 am			
11:30-11:45 am			
10:45-12:00 pm			
12:00-12:15 pm			
12:15-12:30 pm		Lunch	
12:30-12:45 pm			Lunch
12:45-1:00 pm			
1:00-1:15 pm			
1:15-1:30 pm		New Tools and Approaches for Integrated Pest Management I	
1:30-1:45 pm			Sea Louse Biology II
1:45-2:00 pm			
2:00-2:15 pm			
2:15-2:30 pm			
2:30-2:45 pm			
2:45-3:00 pm			
3:00-3:15 pm		Break	
3:15-3:30 pm			Break
3:30-3:45 pm		New Tools and Approaches for Integrated Pest Management II	
3:45-4:00 pm			Sea Louse Biology III
4:00-4:15 pm			
4:15-4:30 pm			
4:30-4:45 pm			
4:45-5:00 pm			
5:00-5:15 pm	Registration		
5:15-5:30 pm			
5:30-6:00 pm	Reception 5:30-9:30 pm		
6:00-6:30 pm			
6:30-9:00 pm			
9:00-9:30 pm			

	Wednesday	Thursday	Friday
	September 3, 2014	September 4, 2014	September 5, 2014
7:00-8:00 am		Breakfast	Breakfast
8:00-8:15 am		Plenary: Karin Boxaspen	Host Immune Responses and Sea Louse Immunomodulation
8:15-8:30 am			
8:30-8:45 am			
8:45-9:00 am			
9:00-9:15 am		Use of Chemotherapeutants, Modes of Action, Targets, and Resistance I	
9:15-9:30 am			
9:30-9:45 am			
9:45-10:00 am			
10:00-10:15 am			
10:15-10:30 am		Break	Break
10:30-10:45 am			
10:45-11:00 am		Use of Chemotherapeutants, Modes of Action, Targets, and Resistance II	Plenary: Geoff Boxshall
11:00-11:15 am			
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10:45-12:00 pm			Conference Wrap Up
12:00-12:15 pm			
12:15-12:30 pm		Lunch	
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1:15-1:30 pm		Sea Lice in Wild and Farmed Fish Populations	
1:30-1:45 pm			
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3:00-3:15 pm		Break	
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3:30-3:45 pm		Poster Session	
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6:30-9:00 pm		Conference Banquet & Dance 6:00 pm-12:00 am	
9:00-9:30 pm			
9:00-12:00 am			

The Organizing Committee for Sea Lice 2014

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Sarah Turner
University of Maine, Maine Aquaculture and Aquatic Animal Health Laboratory, Orono, Maine, 04469, USA

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10:45 Evaluation of organised delousing in a small ecosystem
K. Eliasen*,

11:00 The surveillance and control programme for resistance to chemotherapeutants in Lepeophtheirus salmonis in Norway
R. Grøntvedt, P.A. Jansen*, A. Tarpai, K.O. Helgesen, T.E. Horsberg

11:15 The effect of synchronized fish farm fallowing: experiences from Hardangerfjord 2010-2013
R.M. Serra-Llinares*, P.A. Bjørn, U. Lindstrøm, I.A. Johnsen, A.D. Sandvik, R. Nilsen, B. Finstad, J. Skardhamar, A.D. Sandvik, L. Asplin.

11:30 Sea lice in Chile: historical analysis of surveillance and control programs
R. Ibarra*, A. Tello, J. Campisto and M. H. Medina

11:45 Coordinated sea lice (Caligus rogercresseyi) control plan in Chile 2013-2014
O. Gárate, J. Leal, A. Cariman, A. Guzmán, D. Woywood*

12:00 Evaluating the effect of delousing treatment coordination on the sea lice abundance in southern Chile
G.A. Arriagada*, H. Stryhn, R. Vanderstichel, E.E. Rees, J. Sanchez, J.L. Campistó, R. Ibarra, M. Medina, S. St-Hilaire.

New Tools and Approaches for Integrated Pest Management I

1:15 In-feed solutions for sea lice control: additional tools for integrated pest management
Simon Wadsworth*, Stanko Skugor, Helle Holm, Ragna Heggebo, Jorge Pino, Anne Kari Osmo

1:30 Effects of in feed masking compounds on olfactory and immune response genes of Caligus rogercresseyi settlement in Atlantic salmon (Salmo salar)
Jorge Pino*, Andrés Quiroz, Christopher Hawes, Simon Wadsworth, Gustavo Núñez-Acuña and Cristian Gallardo-Escárate

1:45 Effects of anti-attachment bioactive compounds on the inhibition of frontal filament development in Caligus rogercresseyi copepodids
Jorge Pino Marambio*, Christopher Hawes, Veronica Osorio, Tirza Valenzuela, Simon Wadsworth

2:00 Effects of a salmon semiochemical on infestation by Lepeophtheirus salmonis copepodids in salmon smolts: Preliminary results
P. Pageat* and C. Delfosse

2:15 Evidence of the effect of S.C.A.I.S. (Sealice Copepodid Attachment Inhibiting Semiochemical) on the infesting behavior of Lepeophtheirus
C. Delfosse* and P. Pageat

2:30 Sequential treatment of Atlantic salmon (Salmo salar) with SLICE® (emamectin benzoate) and hydrogen peroxide for the control of salmon lice
D. Morris*, C. Gould , B. Roy , D. Bassett , D. Cockerill

2:45 Improvements in sealice tarpaulin treatment technology using PARAMOVE® (50% Hydrogen Peroxide)
R. Strom*, I.Armstrong, H. Mitchell, P. Astudillo

New Tools and Approaches for Integrated Pest Management II

3:30 Quantitative genetics of susceptibility of Atlantic salmon to the salmon louse
B. Gjerde*

3:45 Changing the host-parasite dynamic with fish farming: behavioural interaction of salmon and sea lice in a cage environment
S. Bui*, F. Oppedal, L.H. Stien, T. Dempster

4:00 Host-parasite mismatch reduces sea lice infestation in farmed salmon through snorkel cage designs
F. Oppedal*, L.H. Stien, S. Bui and T. Dempster

4:15 Shielding skirt for prevention of salmon lice (Lepeophtheirus salmonis) infestation on Atlantic salmon (Salmo salar L.) In cages – effects on cage and cage environment
A.M. Lien*, K. Frank

4:30 Plankton nets as a preventive tool to reduce sea lice (Lepeophtheirus salmonis) infestations in salmon farming
R.N. Grøntvedt*, M.B. Næs, A.B. Kristoffersen and B. Johansen

4:45 Lumpfish: Optimizing the use of a new cleaner fish
A. Johannesen* and R. Arge

5:00 Delousing efficiency of farmed ballan wrasse (Labrus bergylta) against Lepeophtheirus salmonis infecting Atlantic salmon post-smolts
E. Leclercq* and H. Migaud

5:15 Natural sea lice mitigation at an Atlantic salmon (Salmo salar) farm in British Columbia, Canada, using cultured filter-feeding bivalves
A. Byrne*, C.M. Pearce, S.F. Cross, S.R.M. Jones and S.M.C. Robinson

Tuesday
Epidemiology, Modeling and Analysis

9:00 Towards an assessment of regional salmon lice infection pressure
L. Asplin*, P.A. Bjørn, I.A. Johnsen, A.D. Sandvik, J. Skardhamar, J. Albretsen, B. Ådlandsvik, M.S. Myksvoll, R. Nilsen, R. M. Serra-Llinares and U. Lindstrøm

9:15 The predictability of sea lice density in Norwegian fjords
Anne D. Sandvik*, Jofrid Skarðhamar, Pål-Arne Bjørn, Lars Asplin, Serra Rosa Maria Llinares and Ingrid A. Johnsen

9:30 Using a biologically assessed sea lice transport model to determine dispersal characteristics for informing management
N.K.G. Salama*, C.C. Pert, A.G. Murray, I.S. Wallace, J. Dunn, J.G. Fraser, B. Rabe and C.M. Collins

9:45 Modeling dispersion of Caligus rogercresseyi from 102 salmon farm sites in Chile using a 3D hydrodynamic model (SINMOD) and displaying the results in an innovative user-friendly interface
J.E. Unibazo*, Ø. Knutsen and D. Slagstad

10:00 Modelling the influence of wild salmon on the evolution of resistance to chemotherapeutants in sea lice (Lepeophtheirus salmonis)
G. F. McEwan*, M. L. Groner, C. W. Revie, M. D. Fast

Sea Louse Biology I

11:00 The salmon louse life cycle: How did two chalimus stages become four chalimus stages?
C. Eichner, L.A. Hamre*, R. Skern-Mauritzen and F. Nilsen

11:15 Use of RAD sequencing to isolate a sex-specific SNP marker in the salmon louse *Lepeophtheirus salmonis* (Krøyer, 1837)
S. N. Carmichael, M. Bekaert, J. B.Taggart, J.H. Ireland, H. R. L. Christie, D. I. Bassett, J. E. Bron, P. J. Skuce, K. Gharbi, R. Skern-Mauritzen, A. Sturm*

11:30 Characterization of sex determination genes in salmon louse, Lepeophtheirus salmonis
M. Furne, C. M. A. Caipang, R. Skern-Mauritzen

11:45 Assessing the use of ATP as a condition index in the sea louse, Lepeophtheirus salmonis, in the Bay of Fundy
S.M.C. Robinson*, T.R. Lander, K.P. Ang

12:00 Nuclear receptors in salmon lice, Lepeophtheirus salmonis
R. Male*, M. Dondrup, I. Tolås, M. Khatri, K. Gravdal, P. Battachan, F. Nilsen

12:15 Characterization of salmon louse Lepeophtheirus salmonis, genes containing fibronectin type II domains
E. Harasimczuk, F. Nilsen, A. C. Øvergård, S. Grotmol, H. Kongshaug, S. Dalvin

Sea Louse Biology II

1:30 Digestion and reproduction are inhibited by RNA interference mediated knockdown of LsKDELR and LsCOPB2 in the salmon louse
C. Trösße*, F. Nilsen and S. Dalvin

1:45 Identification of an intracellular cystatin in L. salmonis subsp. salmonis and its putative role the digestive process and immune responses
C.M.A. Caipang, S. Mæhle, E.P. Garcia and R. Skern-Mauritzen*

2:00 Molecular characterisation of the ecdysone receptor (EcR) in the salmon louse, Lepeophtheirus salmonis
L. Sandlund* , F. Nilsen, R. Male, H. Kongshaug, S. Grotmol and S. Dalvin

2:15 Identification and characterisation of Halloween genes in sea lice: investigating a novel group of drug targets
C.M. McNair*, J.H. Ireland, Q. Zhong, S.J. Monaghan and J.E. Bron

2:30 Molecular characterization of a salmon louse (Lepeophtheirus salmonis) chitinase using RNA interference in planktonic stages
C. Eichner, E. Harasimczuk, S. Grotmol, F. Nilsen, S. Dalvin*

2:45 The ABC gene family of the salmon louse (*Lepeophtheirus salmonis*)
S.N. Carmichael, J. Heumann, J.E. Bron, M. Bekaert and A. Sturm*

3:00 Recent advances in the production and implementation of farmed ballan wrasse (*Labrus bergylta*) in the Scottish salmon industry
E. Leclercq, B. Grant, A. Chalaris, A. Davie and H. Migaud*

Sea Louse Biology III

3:45 LiceBase: Model organism database and functional genomics tools for the sea lice research community
M. Dondrup*, C. Andreetta, I. Jonassen, F. Nilsen

4:00 Sublethal threshold of Caligus rogercresseyi (Boxshall & Bravo 2000) on the physiological response of the host Salmo salar (Linnaeus 1758)
M.P. González*, L. Vargas-Chacoff and S.L. Marín

4:15 Caligus rogercresseyi transcriptome: Novel insights for key biological processes during the lifecycle of the salmon louse
C. Gallardo-Escárate, V. Valenzuela-Muñoz, G. Núñez-Acuña, J. Chávez-Mardones, W. Maldonado-Aguayo, A. T. Gonçalves, R. Farlora, D. Valenzuela-Miranda

4:30 Microarray profiling in skin revealed protective mechanisms mediated by feeding plant derived anti-lice bioactives against salmon lice in Atlantic salmon
H. Holm, S. Wadsworth, A.K. Osmo, A. Krasnov, Ø. Evensen, S.Skugor*

4:45 Development of bacterial ‘microbiome-markers’ for salmon microbiota mediated resistance against infection with sea louse, Lepeophtheirus salmonis
S. Leadbeater*, N. Derome, M. Llewellyn, K. P. Ang, F. Powell and J. Elliot

5:00 Detection and quantification of planktonic Lepeophtheirus salmonis by real-time PCR
A. Mols-Mortensen*, G. á Norði, E. Danielsen, Á. Jacobsen, D.H. Christiansen and R. Skern

Thursday

Use of Chemotherapeutants, Modes of Action, Targets, and Resistance I

9:00 Screening of pharmaceutical compounds for effect on preadult salmon lice
S.M. Aaen* and T.E. Horsberg

9:15 Are laboratory bioassays an efficient tool for monitoring Caligus rogercresseyi (Boxshall & Bravo 2000) sensitivity to antiparasitics: Weaknesses and strengths
S.L. Marín*, R. Ibarra, M.H. Medina

9:30 Trends in the success of pyrethroid and organophosphate bath treatments against Caligus rogercresseyi in the Chilean salmon industry
A. Tello*, P. Artacho, R. Ibarra and M. H. Medina

9:45 First report of hydrogen peroxide resistance in salmon lice (Lepeophtheirus salmonis) in Norway
K.O. Helgesen*, H. Romstad, S.M. Aaen and T.E. Horsberg

10:00 Emamectin benzoate field data from Norway from spring 2011 until spring 2014
K. Ulven*, B. Lygren

Use of Chemotherapeutants, Modes of Action, Targets, and Resistance II

10:45 Avermectin treatment for Lepeophtheirus salmonis and effects on salmon immunophysiology
M.D. Fast*, K.E. Fitzpatrick, S.L. Purcell, S.C. Johnson, S. Wadsworth, S.K. Whyte

11:00 Emamectin benzoate resistant Salmon lice (Lepeophtheirus salmonis) show changes in ligand-gated ion channel expression
S.N. Carmichael, J.E. Bron, J.B. Taggart, J.H. Ireland, M. Bekaert, S.T.G. Burgess, P.J. Skuce, A.J. Nisbet, K. Gharbi & A. Sturm*

11:15 Transcriptomics of emamectin benzoate responses in resistant Atlantic and sensitive Pacific salmon lice Lepeophtheirus salmonis
B.J.G. Sutherland*, J.D. Poley, O.O. Igboeli, J.R. Jantzen, M.D. Fast, B.F. Koop, S.R.M. Jones

11:30 Global gene expression analysis of copepodid sea lice (Lepeophtheirus salmonis) drug responses using in vitro bioassays
J.D. Poley*, B.J. Sutherland, S.K. Whyte, O.O.Igboeli, S.L. Purcell, K.E. Fitzpatrick, B.F. Koop, M.D. Fast

11:45 Identification of the mechanism behind resistance against organophosphate (azamethiphos) in salmon lice (Lepeophtheirus salmonis)
KP. Kaur*, K. O. Helgesen, M. J. Bakke, T. E. Horsberg

12:00 Azamethiphos resistance – Frequency of resistance alleles in Norwegian salmon
KP. Kaur, S.M. Aaen, K. O. Helgesen, V. Aspehaug, T. E. Horsberg*

Sea Lice in Wild and Farmed Fish Populations

1:15 Public perceptions and framing of salmon louse issues in Norway, the U.K. and Canada
M. Solberg, S. Dalvin*

1:30 Where are all the sea lice? Searching the wild fish of Cobscook Bay
A. Jensen, M. Pietrak*, S. Barker, G. Zydlewski and I. Bricknell

1:45 Where are all the sea lice? A first glance at sentinel fish in Cobscook Bay
C. Frederick*, M. Pietrak, S. Barker, D. Brady and I. Bricknell

2:00 Identifying variations in the potential infestation pressure from sea lice on wild salmonids in a Scottish salmonid aquaculture region
C.C. Pert*, S.J. Middlemas, C.M. Collins, D. Baum and N.K.G. Salama

2:15 Seasonal changes in the abundance of planktonic *Lepeophtheirus salmonis* and *Caligus elongatus* in a fish farming region in the Faroe Islands
G. á Norði*, K. Eliasen, E. Danielsen, K. Simonsen

2:30 Biology and ecology of sea lice on wild and farmed salmonids in the Strait of Georgia and Johnstone Strait, British Columbia, Canada
S.C. Johnson*, C-E.M. Neville, M. Trudel, and S.R.M. Jones

2:45 Occurrence of sea lice (Copepoda: Caligidae) on marine fishes from Jaramijo, an area with potential for sea-cage aquaculture in Ecuador
F.N. Morales-Serna*, P. Loor-Andrade, V. Caña-Bozada, G.B. Mera-Loor, and E.J. Fajer-Ávila

Friday
Host Immune Responses and Sea Louse Immuno-modulation

8:00 Recombinant vaccine efficacy trials against infectious larval salmon lice stages following intraperitoneal immunisation of Atlantic salmon with 9 vaccine candidates
S.J. Monaghan, C.M. McNair, H.C. McDonald, J.H. Ireland, S. Hamilton, D. Knox, W. Roy, K.D. Thompson, A. Adams, R.H. Richards, P.D. Smith, D. Bassett, C. Matthew, A. Preston, F. Groves, S. Boyd, T. Kanellos, D. Asper, J.E. Bron

8:15 Unravelling the acquired immune response to larval sea lice infections: a serological approach
S.J. Monaghan*, C.M. McNair, C. Metochis, K.D. Thompson, A. Adams, H.C. McDonald, S. Hamilton, D. Knox, R. Richards, P.D. Smith, W. Roy, T. Kanellos, D. Asper, J.E. Bron

8:30 Secretory / excretory products of *Lepeophtheirus salmonis* regulate salmon leukocyte migration in vitro
J.L. Piesz*, I.R. Bricknell, Hernan Pizarro, S.E. Barker

8:45 Tissue models for studying host-parasite interactions with salmon lice *Lepeophtheirus salmonis* (Copepoda, Caligidae)
H.C. McDonald*, A.P. Shinn, K.D. Thompson, K.F. Muir, S.J. Monaghan, C.M. McNair, R.H. Richards, D.P. Knox, S. Hamilton, D. Asper, T. Kanellos, J.E. Bron

9:00 Characterization and knock-down of a putative prostaglandin E synthase found in *Lepeophtheirus salmonis*
C. Eichner*, A. Øvergård, F. Nilsen, S. Dalvin

9:15 Increased susceptibility to infectious salmon anemia virus (ISAv) in *Lepeophtheirus salmonis* – infected Atlantic salmon
S.E. Barker*, J. Covello, D. Bouchard, W. Wolters, S. Purcell, M. Fast, I.R. Bricknell

9:30 Transcriptomic evidence for host-specific feeding responses of *Lepeophtheirus salmonis*
L.M. Braden*, B.J.G. Sutherland, B.F. Koop, S.R.M. Jones

9:45 Profiling the effects of plant derived anti-lice bioactives on salmon louse and Atlantic salmon
S. Skugor*, H. Holm, A.K. Osmo, T. Utne, A. Krasnov, Ø. Evensen, S. Wadsworth

10:00 Development of a vaccine against sea lice
Yamila Carpio González, Claudia García Castillo, Juana Maria Lugo González, Jannel Acosta Alba, Liliana Basabe Tuero, Antonio Morales, Reynold Morales, Osmany Rodrigo, Fidel Herrera, Janet Velazquez, Alexis Machin, Yeny Leal, Mario Pablo Estrada*

Notes

Monday – Plenary Talk

N. Halse

Vice President Communications, Cooke Aquaculture

SEA LICE AND SALMON FARMING - A BUSINESS PERSPECTIVE
The importance of sea lice research to the real world business of growing salmon

The underlying motivation for sea lice research and corporate and government investments into research and innovation is the expanding global demand for healthy, affordable and nutritious seafood. Salmon farmers play a critical role in meeting that demand and as human populations increase and wild fish supply either stagnates or diminishes, fish farmers are poised to play an even greater role in the future. Like all farmers, salmon farmers and their veterinarians need access to adequate management and treatment tools to be able to protect the health of their farm animals during the farming process.

Canadian and US salmon farmers do not have these tools today putting them at a global disadvantage in the marketplace. Farmers in Atlantic Canada and Maine have struggled since 2009 to manage naturally occurring sea lice on their farms without the same treatment tools that are approved in other countries. Because salmon farmers in this region have not had stable access to a variety of approved sea lice treatments and because of rising water temperatures, many farms on the east coast are experiencing an increase in the prevalence of sea lice without adequate tools to deal with the problem.

The situation has led to a number of business and management decisions that could have a long-term impact on the region's salmon farming sector.

In spite of the development of Integrated Pest Management Plans (IPMP), both in Canada and the US, farmers are not able to implement these plans because they lack treatment options. IPMPs combine farm management practices with access to a variety of approved treatments that farmers can use strategically to target various life stages of sea lice while considering environmental constraints such as water temperatures. This approach allows farmers to use the right product or the right measure at the right time, thus reducing the overall amount of treatment used while keeping lice numbers at an acceptable low level. Companies continue to make stocking and management decisions that lead to reduced production, job losses and reduced economic impact.

This presentation will provide details of the sea lice management challenge, the business decisions that are being made and the consequences of those decisions. It will also outline our research priorities along with business initiatives and investments that are being made by Canadian and US companies into innovation, non-chemical sea lice treatments and collaborative solutions. Finally, this Business Perspective will present a challenge to various stakeholders to collaborate in creative and focused ways to implement those solutions.

Notes

Monday – Session 1
Co-ordinated Treatments and Sustainable Production

Session Chairs:
James Bron
Michael Pietrak

Session Titles:

10:30 The role of the management cell in sea lice control in Ireland
F. Kane*, D. Jackson, P. O'Donohoe, T. Mc Dermott, S. Kelly and A. Drumm

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K. Eliasen*,

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R. Grøntvedt, P.A. Jansen*, A. Tarpai, K.O. Helgesen, T.E. Horsberg

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11:30 Sea lice in Chile: historical analysis of surveillance and control programs
R. Ibarra*, A. Tello, J. Campisto and M. H. Medina

11:45 Coordinated sea lice (Caligus rogercresseyi) control plan in Chile 2013-2014
O. Gárate, J. Leal, A. Cariman, A. Guzmán, D. Woywood*

12:00 Evaluating the effect of delousing treatment coordination on the sea lice abundance in southern Chile
G.A. Arriagada*, H. Stryhn, R. Vanderstichel, E.E. Rees, J. Sanchez, J.L. Campistó, R. Ibarra, M. Medina, S. St-Hilaire.

12:15 Lunch sponsored by Cooke Aquaculture

The role of the management cell in sea lice control in Ireland

F. Kane[1]*, D. Jackson[1], P. O'Donohoe[1], T. Mc Dermott[1], S. Kelly[1] and A. Drumm[1]

[1]*Marine Institute, Oranmore, Galway, Ireland.*

A Sea Lice Monitoring Programme for finfish farms in Ireland was instigated in 1991 and expanded to all stocks nationally in 1993. The *Monitoring Protocol No.3 for Offshore Finfish Farms - Sea Lice Monitoring and Control* was formally published in 2000. Data collected from the monitoring programme are provided to the farm and circulated to relevant parties directly. The data are also published annually[1].

Following persistent difficulties in achieving sea lice control targets at certain locations; "*A strategy for the improved pest control on Irish salmon farms*"[2] was published in 2008. This outlined a comprehensive range of measures to provide for enhanced sea lice control. It was developed by both regulators and industry in response to difficulties experienced by farms in maintaining low levels of sea lice infestation. These measures draw on the on-going Single Bay Management process and through a number of recommendations seek to advance the suite of tools necessary for improved sea lice control on farms.

Management Cells are one of the key recommendations and are a central tool in the management of sea lice on an individual farm basis. A Management Cell is a local group comprised of salmon producers, statutory monitors, aquaculture development organisations, the farm's veterinary surgeon, the affected farm and possibly other farms within the bay. It is convened when there is prolonged elevated sea lice infestation, to give advice and agree a way forward.

The Management Cell works by bringing all the relevant expertise to bear on the problem situations in real time, actively engaging the affected farm and ensuring that a high priority is given to dealing with the infestation. It is a proactive and integrated mandatory 'real time' management regime, which vigorously deals with failures to control sea lice on a case-by-case basis[3].

A series of case studies are presented describing the management options employed over a period and their outcomes in terms of sea lice control. To date the Management Cell process has contributed to a downward trend of mean sea lice levels on Irish farms.

References

[1] O'Donohoe P, Kane F, Kelly S, Mc Dermott T, Drumm A & D Jackson 2014. National Survey of Sea Lice (*Lepeophtheirus salmonis* Krøyer and *Caligus elongatus* Nordmann) on Fish Farms in Ireland – 2013. *Marine Institute Irish Fisheries Bulletin No 44. http://hdl.handle.net/10793/955*

[2] Department of Agriculture, Fisheries and Food (Ireland) 2009. *A strategy for the improved pest control on Irish salmon farms*. https://www.agriculture.gov.ie/media/migration/fisheries/aquacultureforeshoremanagement/SeaLiceControlStrategy%20230210.pdf

[3] Jackson D. 2011. Ireland: The development of sea lice management methods. In: *Salmon Lice: An integrated approach to understanding parasite abundance and distribution*. 2011. (Ed: Jones. S & R Beamish). Wiley-Blackwell. ISBN-13978-0-8138-1362-2, ISBN-10: 0-8138-1362-X

Evaluation of organised delousing in a small ecosystem

K. Eliasen[1*,]

[1]*Aquaculture Research Station of the Faroes, Hvalvík, Faroe Islands*

Despite its relatively small size (~10 000 km^2), the Faroe shelf (centered at 62°00´N, 06°47´W) contains a distinct neritic ecosystem surrounded by an oceanic environment. Farmed Atlantic salmon (*Salmo salar*) accounts for approximately 42% of the total export value of the Faroe Islands. Hence, the Faroese economy is highly dependent on the aquaculture industry. The main challenge of the Faroese salmon industry is sea lice, *i.e. Lepeophtheirus salmonis* and *Caligus elongatus*. In spring 2013 the Faroese salmon farming industry initiated a united delousing programme. The results of the organised delousing was very convincing, and the sea lice pressure decreased substantially in the following months. The results suggests that organised delousings are more effective, and that the oceanic features of farming areas ought to be implemented in sea lice management strategies.

The surveillance and control programme for resistance to chemotherapeutants in *Lepeophtheirus salmonis* in Norway

R. Grøntvedt[1], P.A. Jansen[1]*, A. Tarpai[1], K.O. Helgesen[2], T.E. Horsberg[2]

[1]*Norwegian Veterinary Institute, Oslo, Norway;*
[2]*Norwegian University of Life Sciences, School of Veterinary Science , Oslo, Norway*

The Norwegian Veterinary Institute is responsible for carrying out a surveillance and control program to follow the development of resistance traits in salmon lice against different chemotherapeutants. The aim of the program is to provide a description of the spatio-temporal distribution of resistance along the Norwegian coast as a basis for evaluating salmon lice control strategies. The program consists of a passive and an active part. The passive part summarizes register data on the prescription of different substances to control salmon lice infestations, and register data reported from salmon farms regarding treatment failures and bioassay results. The active part of the surveillance program comprises bioassay testing for sensitivity of salmon lice to the substances emamectin benzoate, deltamethrin (pyrethroid) and azamethiphos, covering the areas of salmon farm production in Norway. The bioassays were of a new simplified design[1] and they were carried out by local fish health services. Each bioassay test consisted of a low concentration test for lice sensitivity and a high concentration test to simulate treatment effects. From the passive part, we find that prescriptions of pyrethroids have increased over the years 2011 - 2013, whereas prescriptions of emamectin benzoate have decreased. Maps showing the distribution of drug prescriptions will be presented. In the active part, 145 simplified bioassay tests on sea lice from 62 salmon farm locations were performed. The results from testing of azamethiphos and deltamethrin were similar. For low concentration tests, the only area with salmon lice test-mortalities exceeding 80 % (indicative of fully sensitive populations) was in Finnmark County in the far north. Low lice mortalities in high concentration tests were found especially in the areas of northern Nordland/southern Troms, Trøndelag and Hordaland. There seemed to be spatial correlations between the distribution of prescription data and bioassay test results for azamethiphos and pyrethroids. Bioassay test results for emamectin benzoate were less conclusive than for azamethiphos and pyrethroids, which may have to do with the choice of concentrations in the tests. We conclude that reduced sensitivity in salmon lice to common chemotherapeutants is widespread along the Norwegian coast and that areas with fully sensitive lice coincide with low treatment intensity.

References:
[1]K.O. Helgesen & T.E. Horsberg 2013. Single-dose field bioassay for sensitivity testing in sea lice, *Lepeophtheirus salmonis*: development of a rapid diagnostic tool. J. Fish Dis. 36:3 261-272.

The effect of synchronized fish farm fallowing: experiences from Hardangerfjord 2010-2013

R.M. Serra-Llinares*[1], P.A. Bjørn[1], U. Lindstrøm[1], I.A. Johnsen[1], A.D. Sandvik[1] R. Nilsen[1], B. Finstad[2], J. Skardhamar[1], A.D. Sandvik[1], L. Asplin[1].

[1]*Institute of Marine Research, Bergen, Norway.*
[2]*Norwegian Institute for Nature Research, Trondheim, Norway.*

In 2010 The Norwegian Food Safety Authority implemented a huge action plan against salmon lice which included the synchronized fallowing of fish farms in geographically restricted areas, with the main aim of preventing salmon lice having population reducing effects on wild salmonids. This paper investigates the effect of the management measures taken with regard to this goal and analyzes spatiotemporal changes in infection pressure in the period 2010-2013 in Hardangerfjord, Norway. Production patterns and lice abundance on farmed fish were investigated before and during the synchronized fallowing of farms, changes on the lice infection pressure along the fjord were examined by use of sentinel cages and the effect on wild populations of salmonids was investigated by counting lice on wild caught sea trout and out-migrating salmon smolts. Last, a salmon lice dispersion model was used to simulate the spread of lice from fish farms in Hardangerfjord based on real lice numbers. Our results indicate that, whereas the synchronized fallowing resulted in very low numbers of lice produced during spring and early summer in fallowed areas, the infection pressure could remain high due to the transport of lice from other sources. Fallowing farms in the outer part of the fjord had a positive effect for wild salmonids both in the local and global scale, whereas fallowing farms in the middle part of the fjord tended to increase the lice infection pressure in the whole length of the fjord. Lice dispersal simulations highlighted the role of fish farms in the outer fjord as an important source of lice for other areas.

Sea lice in Chile: historical analysis of surveillance and control programs

R. Ibarra[1]*, A. Tello[1], J. Campisto[1] and M. H. Medina[1]

[1]*Instituto Tecnológico del Salmón, INTESAL de SalmonChile, Puerto Montt, Chile*

Periodic monitoring of sea lice is an essential tool for controlling the disease in salmon producing countries, allowing companies to take remedial measures aiming at implementing actions for an effective control of this parasitism. In Chile, these monitoring programs started with the one of the salmon grower association (SalmonChile) in 1999 In 2006 INTESAL reinforced this initiative through the implementation of the Zonal Program that aim for coordinated efforts against Caligidosis in areas identified as critical. During 2007, the Program was adopted by the health authority becoming mandatory for the entire industry. In this study the history and impact of these surveillance and control programs were analyzed, with emphasis on the sea lice abundance during the last four years. Results showed that caligus abundance could be explained partly by various control measures, although the impact of each varies with respect to the effect on parasite abundance and production and environmental conditions (fig 1). The program has collected information from many cycles to enable build a solid database at site, neighborhood and country level. Also has been flexible enough to incorporate new knowledge and eliminate actions that have not had the expected effect.

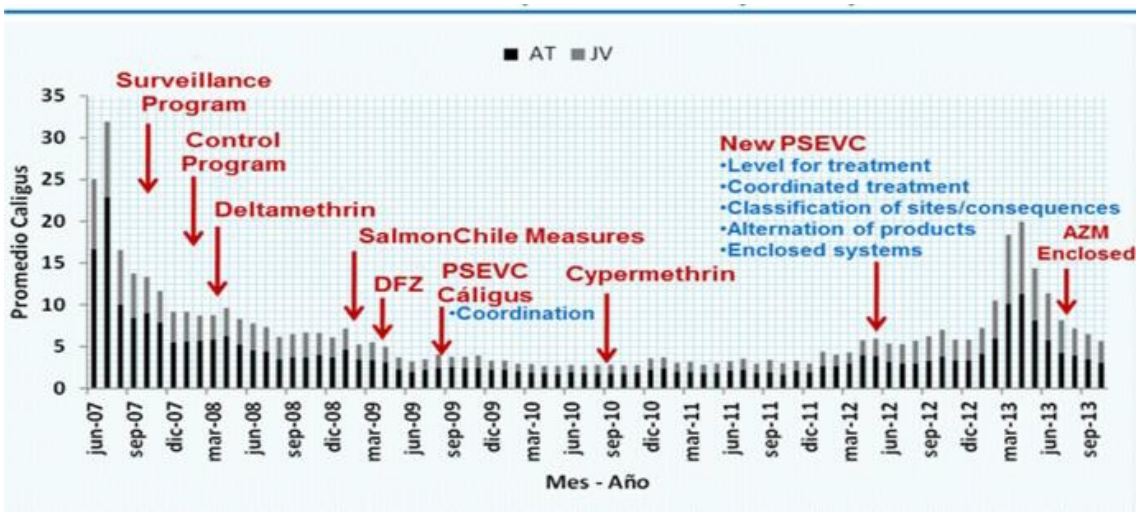

Fig 1: Sea lice abundance in Chile and evolution of control and surveillance actions in Chile (2007-2013).

Coordinated sea lice (*Caligus rogercresseyi)* control plan in Chile 2013-2014

O. Gárate[1], J. Leal, A. Cariman[1], A. Guzmán[1], D. Woywood[1]*

[1]*Aquabench S.A., Puerto Montt, Chile*

Caligus rogercresseyi is the most relevant parasite affecting the salmon industry in Chile, causing high level of stress in fish and consequently increased susceptibility to infectious diseases and causing a significant decline in production.

After the Infectious Salmon Anaemia (ISA) health crisis in 2007, these parasitoses had been kept under control, however, during the summer of 2013 an increase was reported with a mean abundance of 12 *Caligus* across the industry. This required several sea sites to start early harvests as a mitigation measure.

After this high mean abundance of *Caligus* (fig. 1), a *Caligus* Coordinated Control Plan was initiated, which is led by Aquabench. This plan is integrated between 15 salmon producers, equivalent to 87% of the total production in Chile and the main objective is to keep *Caligus* levels under control.

As a result of this project tracking systems for parasite infection levels, treatment window, alternation of anti-parasitic drugs and drug efficacy and sensitivity have been developed. Together with the control of the above-mentioned aspects, important progress has been made in the development of non-drug alternatives, such as cleaner fish, skirts and thermal delousing among others. Standardization of *Caligus* retention filters on wellboats is also currently under development. Finally this project includes studies for the optimization of production areas and improvement of the strategic use of drugs *e.g*. use of coordinated winter treatment.

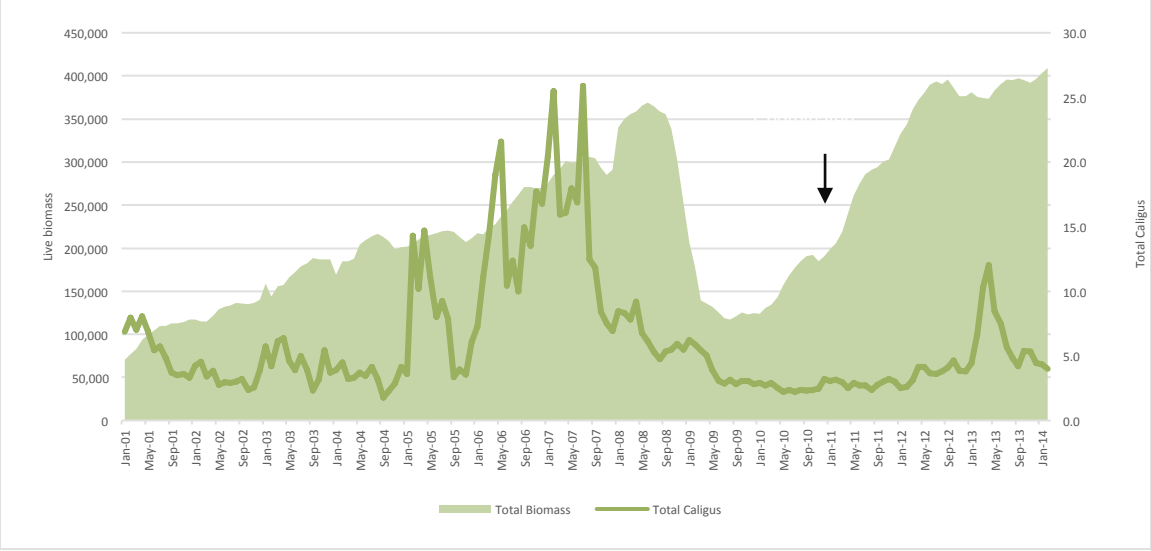

Fig.1: Atlantic salmon and rainbow trout total live biomass vs total *Caligus* in Chile, 2001 -2014 (Data source: Sernapesca, Intesal and Aquabench)

Evaluating the effect of delousing treatment coordination on the sea lice abundance in southern Chile

G.A. Arriagada[1]*, H. Stryhn[1], R. Vanderstichel[1], E.E. Rees[1], J. Sanchez[1], J.L. Campistó[2], R. Ibarra[2], M. Medina[2], S. St-Hilaire[1].

[1]*University of Prince Edward Island, Charlottetown, PE, Canada.*
[2]*Instituto Tecnológico del Salmón, Puerto Montt, Chile.*

Treatment coordination is a management strategy used to control sea lice infection in most of the salmon-producing countries. However, there is no evidence that confirms the beneficial effect of treatment coordination, nor at what extent of the temporal and spatial scales of the coordination is more efficient. The objective of this observational study was to evaluate the effect of coordination of treatments on *Caligus rogercresseyi* levels up to 8 weeks after a single immersion treatment procedure in southern Chile. We modeled the adult sea lice mean abundance at the farm level in a weekly basis from 2 to 8 weeks after a single immersion treatment based on a set of predictor variables, using linear mixed effect models. We tested the effect of the coordination efficacy, expressed as the number of neighboring farms that reported immersion treatment within a given coordinating window, on our response variable in 16 different scenarios (*i.e.* coordinating windows) defined by the combination of four time periods (1, 2, 3 and 4 weeks) and four distances (10, 20, 30 and 40 km). Akaike Information Criteria (AIC), coefficient magnitudes, and predicted adult lice mean abundances were considered for model comparison. Treatment efficacy was significantly associated with reduced adult lice levels between week 4 to 7 after a single immersion treatment, depending on the temporal and spatial extent of the coordinating window. The effect seemed to last more weeks and had stronger coefficients with shorter time periods and closer distances.

Notes

Notes

Monday – Session 2
New Tools and Approaches for Integrated Pest Management I

Session Chairs:
Julia Mullins
Jorge Pino

Session Titles:

1:15 In-feed solutions for sea lice control: additional tools for integrated pest management
Simon Wadsworth*, Stanko Skugor, Helle Holm, Ragna Heggebo, Jorge Pino, Anne Kari Osmo

1:30 Effects of in feed masking compounds on olfactory and immune response genes of Caligus rogercresseyi settlement in Atlantic salmon (Salmo salar)
Jorge Pino*, Andrés Quiroz, Christopher Hawes, Simon Wadsworth, Gustavo Núñez-Acuña and Cristian Gallardo-Escárate

1:45 Effects of anti-attachment bioactive compounds on the inhibition of frontal filament development in Caligus rogercresseyi copepodids
Jorge Pino Marambio*, Christopher Hawes, Veronica Osorio, Tirza Valenzuela, Simon Wadsworth

2:00 Effects of a salmon semiochemical on infestation by Lepeophtheirus salmonis copepodids in salmon smolts: Preliminary results
P. Pageat* and C. Delfosse

2:15 Evidence of the effect of S.C.A.I.S. (Sealice Copepodid Attachment Inhibiting Semiochemical) on the infesting behavior of Lepeophtheirus
C. Delfosse* and P. Pageat

2:30 Sequential treatment of Atlantic salmon (Salmo salar) with SLICE® (emamectin benzoate) and hydrogen peroxide for the control of salmon lice
D. Morris*, C. Gould , B. Roy , D. Bassett , D. Cockerill

2:45 Improvements in sealice tarpaulin treatment technology using PARAMOVE® (50% Hydrogen Peroxide)
R. Strom*, I.Armstrong, H. Mitchell, P. Astudillo

3:00 Break sponsored by Solvay

In-feed solutions for sea lice control: additional tools for integrated pest management

Simon Wadsworth[1]*, Stanko Skugor[2], Helle Holm[2], Ragna Heggebo[1], Jorge Pino[1], Anne Kari Osmo[1]

[1]*EWOS Innovation, Dirdal, 4335, Norway*
[2]*Sea Lice research Centre, NMBU, Norway*

Improved efficacy has been achieved by co-ordinating treatments of the insect growth regulator, diflubenzuron (Releeze) with azamethiphos (Salmosan). By co-ordinating treatments, all stages of sea lice were targeted and effectively removed. Further improvements can be made by co-ordinating additional medicines, such as emamectin benzoate (SLICE), to reduce re-infection and increase the interval between treatments.

Additional tools for sea lice control can be implemented to support the use of these treatment regimes. Sea lice have advanced olfactory and contact chemoreceptors that are required for accurate identification of host-specific molecules. Both *Lepeophtheirus salmonis* and *Caligus rogercresseyi* exhibit a number of responses to specific compounds (kairomones) present in salmon mucus. These responses may be inhibited if non-host compounds are detected. Recent studies have shown that specific plant based compounds can effectively disrupt the settlement and attachment process of sea lice on Atlantic salmon. By deploying specific plant-based compounds in feed, these anti-attachment controls can reduce lice settlement and increase the interval between treatments.

Feed management methods can effectively maintain fish at depth and allow them to remain out of the surface layers. This has been correlated to reduced sea lice infection. A new method incorporating sub-surface feeders has been developed to feed fish at depth. This has resulted in a significant reduction in sea lice numbers.

These dietary controls tools (medicines, functional feeds and feed management) will be discussed within an overall integrated pest management approach.

E-mail address: simon.wadsworth@ewos.com

Effects of in feed masking compounds on olfactive and immune response genes of *Caligus rogercresseyi* settlement in Atlantic salmon (*Salmo salar*)

Jorge Pino[1]*, Andrés Quiroz[3], Christopher Hawes[1], Simon Wadsworth[2], Gustavo Núñez-Acuña[4] and Cristian Gallardo-Escárate[4]

[1]*EWOS Innovation Chile. Pargua KM57 Colaco KM5 Puerto Montt, Chile.*
[2]*EWOS Innovation AS, Dirdal, 4335 Dirdal, Norway.*
[3]*Departamento de Ciencias Químicas y Recursos Naturales, Universidad de La Frontera, Temuco, Chile.*
[4]*Interdisciplinary Center for Aquaculture Research, Universidad de Concepción, Concepción, Chile.*

Sea lice use a series of host molecules to find, identify their fish hosts. Due to their masking effect against insects, we considered different plant-derived compounds as good candidates to be included in feeds for Atlantic salmon as part of preventive measures against the growing problem of lice infestations in farmed salmonids. The aim of this study was to evaluate the effect of masking compounds and immunostimulants, applied in salmon diets, in the infestation level of *Caligus rogercresseyi*. Atlantic salmon groups, infected with salmon louse, were fed with five diets comprising different concentration of masking compound and immunostimulants. Salmon louse samples were taken 3 days after the infestation. Diets contained between 1 and 3% of the bioactive compounds. Four olfactory ionotropic receptors and three immune-related genes were evaluates. Every gene increased its expression levels at 3% of masking compounds plus the incorporation of the immunostimulant, except by the Akirin gene. Furthermore, tank challenge results indicated that level of infestation of *C. rogercresseyi* was reduced between 15 and 22 % compared with the Control after 3 week of feeding treatment. These results suggest a response of the parasite in the presence of the modified diets. Furthermore, since the modified diet was effective to decrease infestation level, could improve the sanitary level of actual status of Chilean salmon industry.

Effects of anti-attachment bioactive compounds on the inhibition of frontal filament development in *Caligus rogercresseyi* copepodids

Jorge Pino Marambio[1]*, Christopher Hawes[1], Veronica Osorio[1], Tirza Valenzuela[1], Simon Wadsworth[2]

[1]*EWOS Innovation Chile, Camino Pargua, Colaco, Puerto Montt, Chile*
[2]*EWOS Innovation AS, Dirdal, 4335 Dirdal, Norway.*

Sea lice use a series of specific molecules to locate, identify and attach to their fish hosts. Once lice have located and positively identified their host, they can start the attachment process. The frontal filament plays an important role as primary attachment device of chalimus and pre-adult stages. Anti-attachment compounds have shown to be effective at reducing settlement of lice due to their repellent and toxic properties that interfere with host recognition and attachment. A range of bioactive plant based products have been documented against reducing lice settlement and attachment. *In vitro* testing has shown that a plant based bioactive compound had an effect on inhibiting the development of the frontal filament. Copepodids were collected and placed in agar-mucus plates with and without anti-attachment compounds within a dose dilution study design. Different concentration of anti-attachment added in the agar-mucus plates showed different levels of frontal filament development. None of these compounds allowed full development of the frontal filament compared with control. The results *correlated closely with further in vivo assessments. This type of in* vitro evaluation will allow us to screen and identify new anti-attachment compounds with a potential to control sea lice infestation.

Effects of a salmon semiochemical on the infestation by *Lepeophtheirus salmonis* copepodids in salmon smolts: Preliminary results

P. Pageat[1]* and C. Delfosse[1]

[1]*Research Institute in Semiochemistry and Applied Ethology, Saint-Saturnin-Lès-Apt, France.*

The purpose of this study is to assess the existence of semiochemicals inhibiting the attachment of copepodids on salmon.

The identification of putative copepodid attachment inhibitors, was managed by analyzing the cutaneous mucus obtained from farmed salmon and rainbow trout. Three groups of fish were defined: infested fish, non-infested fish obtained from infested pens, and non-infested fish obtained from non-infested farms. The mucus was analyzed by GC-GC-MS method: 7 putative compositions were identified.

The effects of the different compositions were assessed using a standardized infestation test. Smolts obtained from farms were treated with a 6ppm solution of the tested composition for 10 minutes and then continuously bathed with a 3ppm solution for the remainder of the test period. A reference group was used for each composition, and was treated with a solution of the solvent (ethyl-alcohol) following the same protocol. During the test, they were exposed to 60 copepodids for 45 min., they were then anaesthetized and euthanized. The entire body surface was then scrubbed to get the mucus and the copepodids. This material was filtered and the copepodids were counted. The gills were also dissected to count the attached copepodids.

The reference groups were infested by 21.2 ± 3.8 copepodids on the body; 3 ± 1.8 on the gills. Between the tested compositions, only one (encoded SCAIS) showed a significantly lower infestation on the body with 8 ± 2.8 copepodids; there was no difference regarding the infestation on the gills.

This semiochemical appears as a promising product for further development of infestation inhibitors.

Evidence of the effect of S.C.A.I.S. (sealice copepodid attachment inhibiting semiochemical) on the infesting behavior of *Lepeophtheirus salmonis* copepodids in salmon smolts: Preliminary results

C. Delfosse[1]* and P. Pageat[1]

[1]*Research Institute in Semiochemistry and Applied Ethology, Saint-Saturnin-Lès-Apt, France.*

The S.C.A.I.S. was identified from our previous studies. The purpose of this paper is to present the validation of this semiochemical during a standardized infestation test, with a positive reference group (non-treated salmon) and a negative group (Atlantic cod *Gadus morhua*).

Smolts obtained from farms, were treated with a 6ppm SCAIS solution, for 10 min, and then continuously bathed with a 3ppm solution for the remainder of the test. The control group was treated with the solvent (ethyl-alcohol) following the same protocol. The cod, of a weight comparable with that of the smolts, were obtained from local fishermen. During the test, all fish were exposed to 60 copepodids for 45 min., they were then euthanized (Benzoak®). The entire body surface was scrubbed to get the mucus; this material was filtered and the copepodids counted. The gills were dissected to count the attached copepodids. Data were analyzed using one-way ANOVA.

The control salmon (n=14) were infested by 20.8 ± 3.4 copepodids on the body; 3.3 ± 2.1 on gills. The SCAIS salmon (n=16) showed a significantly lower infestation on the body with 6.3 ± 2.6 copepodids, and the cod (n=7) had 4.7 ± 2.3 copepodids. There was no significant difference between SCAIS salmon and cod. There was no difference regarding the infestation on gills between the 3 groups.

This semiochemical appears to inhibit stable attachment to salmon, leading to a copepodid infestation comparable to non-host fish species. Further study should assess if the copepodids attached in such a context, remain attached and become chalimus.

Sequential treatment of Atlantic salmon (*Salmo salar*) with SLICE® (emamectin benzoate) and hydrogen peroxide for the control of salmon lice (*Lepeophtheirus salmonis*)

D. Morris[1]*, C. Gould [1], B. Roy [2], D. Bassett [2], D. Cockerill [3]

[1] *MSD Animal Health, Milton Keynes, UK*
[2] *MERL, IOA Stirling, UK*
[3] *Marine Harvest Scotland, UK*

SLICE® (emamectin benzoate) is a medicated feed premix indicated for the treatment and prevention of sea lice infestations in salmon. When fed at the standard dose rate of 50µg emamectin benzoate (EB)/kg body weight/day for 7 consecutive days, SLICE kills all parasitic stages of sea lice. Since its' introduction over a decade ago, SLICE has established a proven record of reliable field performance and thus become the leading product for sea lice control. Hydrogen peroxide (H_2O_2), registered as Interox™ Paramove 50™ (Solvay Chemical International S.A.), is licensed for the bath treatment of salmon suffering from infestation with motile (pre-adult to adult) sea lice. Hydrogen peroxide has been used widely as part of sea lice prevention programs.

Following integrated pest management guidelines, the industry has been looking at ways to improve sea lice control utilizing the range of available registered products. The sequential use of SLICE and hydrogen peroxide has been employed to improve sea lice control, particularly in areas where sea lice populations are showing reduced sensitivity to one or more of the registered sea lice treatments.

To further evaluate the use of this sequential treatment approach, a laboratory study was conducted using an EB-resistant strain of sea lice. This demonstrated that the sequential use of SLICE followed by hydrogen peroxide at either 7 days or 12 days post-SLICE results in good clearance and subsequently reduced settlement of EB-resistant strains of sea lice for at least 39 days after the end of the SLICE treatment.

Improvements in sealice tarpaulin treatment technology using PARAMOVE® (50% Hydrogen Peroxide)

R. Strom[1]*, I.Armstrong[2], H. Mitchell[3], P. Astudillo[4]

[1]*Aqua Pharma, Lillehammer, Norway*
[2]*Aqua Pharma, Inverness, Scotland*
[3]*Aqua Pharma, Kirkland, WA, USA*
[4]*Aqua Pharma, Puerto Montt, Chile*

PARAMOVE® is a special grade of hydrogen peroxide developed by Solvay for the fish farming industry. The Global Salmon Initiative has recently recognised hydrogen peroxide as part of its best practice treatment for salmon lice control. Working together Solvay and Aqua Pharma provide a comprehensive and reliable logistics package for customers. Aqua Pharma works closely with PARAMOVE® customers to ensure safe delivery and efficient dosing into full enclosure tarpaulins and well boats.

The development of PARAMOVE® treatment technology has been critical for maximizing safety and efficacy for the salmon farmer. In 2009, Aqua Pharma successfully introduced PARAMOVE® ISO tank dosing systems. Aqua Pharma's technical expertise, combined with the stewardship standards of Solvay as manufacturer and Marketing Authorization holder, has enabled our partnership to successfully deliver safe use for people and for fish under veterinary care.

Building steadily on our operational experience in Europe and North America, significant advances have recently been made in tarpaulin treatment technology. In particular, our large cage expertise in Norway has delivered significant improvements in treatment technology.

The therapeutic index of hydrogen peroxide remains narrow, and lice populations can become accustomed to any product used too intensively or sub optimally. The use of proven technology greatly assists the achievement of reliable and consistent results, necessary for the use of PARAMOVE® as an integral part of an Integrated Pest Management strategy.

This presentation will update the participants of Sealice 2014 on these technological advances. Such operationally proven technology from Aqua Pharma can assist the sustainability of our industry.

Notes

Notes

Monday – Session 3
New Tools and Approaches for Integrated Pest Management II

Session Chairs:
Randi Grøndveldt
Eric Leclercq

Session Titles:

3:30 Quantitative genetics of susceptibility of Atlantic salmon to the salmon louse
B. Gjerde*

3:45 Changing the host-parasite dynamic with fish farming: behavioural interaction of salmon and sea lice in a cage environment
S. Bui*, F. Oppedal, L.H. Stien, T. Dempster

4:00 Host-parasite mismatch reduces sea lice infestation in farmed salmon through snorkel cage designs
F. Oppedal*, L.H. Stien, S. Bui and T. Dempster

4:15 Shielding skirt for prevention of salmon lice (Lepeophtheirus salmonis) infestation on Atlantic salmon (Salmo salar L.) In cages – effects on cage and cage environment
A.M. Lien*, K. Frank

4:30 Plankton nets as a preventive tool to reduce sea lice (Lepeophtheirus salmonis) infestations in salmon farming
R.N. Grøntvedt*, M.B. Næs, A.B. Kristoffersen and B. Johansen

4:45 Lumpfish: Optimizing the use of a new cleaner fish
A. Johannesen* and R. Arge

5:00 Delousing efficiency of farmed ballan wrasse (Labrus bergylta) against Lepeophtheirus salmonis infecting Atlantic salmon post-smolts
E. Leclercq* and H. Migaud

5:15 Natural sea lice mitigation at an Atlantic salmon (Salmo salar) farm in British Columbia, Canada, using cultured filter-feeding bivalves
A. Byrne*, C.M. Pearce, S.F. Cross, S.R.M. Jones and S.M.C. Robinson

Quantitative genetics of susceptibility of Atlantic salmon to the salmon louse

B. Gjerde[1]*

[1]*Nofima, N-1430 Ås, Norway*

Current control methods for the salmon louse are all short term since the fish may be re-infected and thus have to be deloused at regular intervals. Susceptibility to the louse shows substantial genetic variation both based on field (Kolstad, *et al*., 2005) and controlled challenge test (Gjerde *et al*., 2010; Gjerde *et al*., 2011) data. High genetic correlation between lice count per fish during challenge test and natural infection (Kolstad *et al*., 2005; Gjerde *et al*., 2014), and between sessile and motile/pre-adult lice counts (Gjerde *et al*., 2010) facilitate the use of controlled challenge tests in selective breeding for increased lice resistance. The genetic correlations of susceptibility to the louse with both growth and susceptibility to furunculosis, ISA and IPN are all found to be close to zero (Gjerde, 2013). Thus the prospects are good for reducing the need for delousing through selection for increased resistance to the louse. Predictions show that selection for increased resistance only will reduce lice count per fish with 24 % per generation and 75 % over five generations. However, strict delousing rules and competition between breeding companies makes it difficult for a company to put a lot of weight on this trait relative to other traits in the breeding objective. It may therefore take a long time before a grow-out farmer will see a positive effect in terms of reduced number of delousing events. This obstacle may be overcome by demanding that grow-out farmers only use offspring of parents selected for increased lice resistance.

References:

B. Gjerde, B. Saltkjelvik, & J. Ødegård 2010. Quantitative genetics of salmon lice resistance in Atlantic salmon at different life stages. In: Proc. of the 9th WCGALP, Leipzig, Germany, 1-6 August.

B. Gjerde, J. Ødegård, & I. Thorland 2011. Estimates of genetic variation in the susceptibility of Atlantic salmon (*Salmo salar*) to the salmon louse *Lepeophtheirus salmonis*. *Aquaculture 314, 66–72.*

B. Gjerde 2013. Towards selection for increased resistance to the sea lice in Atlantic salmon. Nofima report 16/2013.

K. Kolstad, P. A. Heuch, B. Gjerde, T. Gjedrem, & R. Salte 2005.Genetic variation in resistance of Atlantic salmon (*Salmo salar*) to the salmon louse *Lepeophtheirus salmoni. Aquaculture 247: 145-151.*

Changing the host-parasite dynamic with fish farming: behavioural interaction of salmon and sea lice in a cage environment

S. Bui[1]*, F. Oppedal[2], L.H. Stien[2], T. Dempster[1]

[1]*University of Melbourne, Victoria, Australia*
[2]*Institute of Marine Research, Matredal, Norway*

Prior to the advent of salmon farming, the parasitic sea lice and its salmonid host typified a co-evolved host-parasite system. Out-migrating salmonids exhibit behaviours to avoid parasites and deter attachment (Plantalech Manel-la *et al*. 2009) while conversely, infective sea lice copepodids position themselves to have the greatest chance of encounter with a potential host using mechano- and chemosensory host-finding abilities (Mordue Luntz & Birkett 2009). With modern salmon farming, the host-parasite landscape has suddenly changed. Host abundance has increased drastically through farming to approximately 943 times the wild population (Torrissen *et al*. 2013). The availability of hosts has allowed lice to proliferate at exceptionally fast rates, and consequently the dynamics of the system have shifted.

However, little is known about the new behavioural interactions between host and parasite. Has it remained the same, or are farmed salmon behaviours an adaption to their new environment? We aimed to determine if salmon could exhibit behaviours to avoid lice attachment within the boundaries of a sea-cage. We monitored jumping behaviour, swimming depth and speed of salmon in three sea-cages, while counting lice loads at periodic intervals. Behaviours could then be attributed to the increasing gradient of lice infestation. In parallel, we followed tagged individuals in a sea cage to map their swimming depth when pre-infested with known amounts of sea lice. Preliminary results reveal highly variable behaviours in the school, the mechanisms of which will be discussed in detail. Tagged fish showed altered depth distribution with high lice loads compared to those with no lice, however, these are opposing behaviours to their wild counterparts, lending evidence to adaption to the cage environment.

References

A.J. Mordue Luntz & M.A. Birkett 2009. A review of host finding behaviour in the parasitic sea louse, Lepeophtheirus salmonis (Caligidae: Copepoda). J. Fish Dis. 32:3-13.

N. Plantalech Manel-la, E.B. Thorstad, J.G. Davidsen, F. Økland, R. Sivertsgård, R.S. McKinley, & B. Finstad 2009. Vertical movements of Atlantic salmon post-smolts relative to measures of salinity and water temperature during the first phase of migration. Fisheries Manage. Ecol. 16:147-154.

O. Torrissen, S. Jones, F. Asche, A. Guttormsen, O.T. Skilbrei, F. Nilsen, T.E. Horsberg, & D. Jackson 2013. Salmon lice – impact on wild salmonids and salmon aquaculture. J. Fish Dis. 36:171-194.

Host-parasite mismatch reduces sea lice infestation in farmed salmon through snorkel cage designs.

F. Oppedal[1]*, L.H. Stien[1], S. Bui[2] and T. Dempster[2]

[1]*Institute of Marine Research, Matredal, Norway*
[2]*University of Melbourne, Victoria, Australia*

High-density production of fish within aquaculture creates ideal conditions for parasite outbreaks in farmed fish, with cascading negative effects on wild populations. Under current farming conditions, salmon cannot avoid infections and outbreaks are inevitable. Combining knowledge of the behaviours of the salmon louse and Atlantic salmon, we hypothesized that infection-risky environments existed and could be avoided. Here, we show that new louse infections were repeatedly reduced up to 84% when a depth-related spatial mismatch between host and parasite was induced. The infectious louse stage is phototactic, which concentrates it in surface waters. This behaviour maximizes encounters with salmon that often swim, feed and jump in surface waters. By placing a net roof as a vertical barrier in cages to hold salmon deeper than the parasite-risky surface layer, we reduced louse infections compared to control cages without manipulation. Salmon were still able to access surface waters and express their natural behaviours through a central chamber termed “the snorkel”, that was impermeable to parasites. In a separate experiment, we showed that louse infections were not reduced when no spatial mismatch existed. Our results demonstrate for the first time that severe parasite infections can be reduced by designing fish farms to limit host-parasite encounters, yet still enable fish to exhibit their full behavioural repertoire. We predict that these results will drive major technological innovation of current salmon farming systems to increase salmon welfare and improve production efficiency. If prevention of parasites on farmed fish is more successful, the population-level impacts of parasites on wild fish should also diminish.

Shielding skirt for prevention of salmon lice (*Lepeophtheirus salmonis*) infestation on Atlantic salmon (*Salmo salar* L.) In cages – effects on cage and cage environment

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[1]*SINTEF Fiskeri og havbruk, Trondheim, Norway*

Infestation of salmon lice (*Lepeophtheirus salmonis*) is, besides escape of fish (Naylor *et al*., 2005), currently the main challenge for Norwegian salmon (*Salmo salar* L.) aquaculture. Salmon lice are a serious threat to fish welfare (Costello, 2006), and result in large expenses for the salmon farmers (Costello, 2009).

Higher infestations of salmon lice have been found to develop at the upper parts of the water column (Hevrøy *et al*., 2003). Shielding these upper parts of salmon cages from lice larvae floating with the ocean currents might thus reduce the level of salmon lice infestations significantly. Preliminary tests have shown the shielding skirts' ability to reduce the salmon lice infestation on salmon in cages by a factor of four (Næs *et al*., 2012).

A tarpaulin skirt mounted on the cage will, however, increase the effective solidity of the cage; hence it is necessary to study the resulting cage mooring loads, the net deformation and the water flow through and around the cage to prevent damage to the structures and the fish.

This study consists of model experiments, numerical simulations, and full scale experiments.

The results have shown how a shielding skirt affects the mooring loads on a fish cage, which is important regarding mooring certification. They have also shown how the skirt deforms in different water currents, increasing the understanding of water exchange and lice infestation. In addition, color dye and oxygen measurements in full scale experiments, contribute to a better understanding of the current flow.

Results from the study will be presented.

References

Naylor R., Hindar K., Fleming I.A., Goldburg R., 2005. Fugitive salmon: assessing the risks of escaped fish from net-pen aquaculture. BioScience 55, 427-437.

Costello M.J., 2006. Ecology of sea lice parasitic on farmed and wild fish. Trends in Parasitology 22, 475-483.

Costello M.J., 2009. The global economic cost of sea lice to the salmonid farming industry. Journal of Fish Diseases 32, 115-118.

Hevrøy E.M., Boxaspen K., Oppedal F., Taranger G.L., Holm J.C., 2003. The effect of artificial light treatment and depth on the infestation of the sea louse Lepeophtheirus salmonis on Atlantic salmon (Salmo salar L.) culture. Aquaculture vol. 220, 1-14.

Næs M., Heuch P.A., Mathisen R., 2012. Report: Bruk av «luseskjørt» for å redusere påslag av lakselus Lepeophtheirus salmonis (Krøyer) på oppdrettslaks. Vesterålen fiskehelsetjeneste. In Norwegian.

Plankton nets as a preventive tool to reduce sea lice (*Lepeophtheirus salmonis*) infestations in salmon farming

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[1]*Norwegian Veterinary Institute, Trondheim, Norway*
[2]*Vesterålen Fish Health Service, Sortland, Norway*
[3]*Norwegian Veterinary Institute, Oslo, Norway*
[4]*Nordlaks Farming, Stokmarknes, Norway*

An integrated management approach to controlling sea lice in salmon farming should include efficient non–medical tools. In a field study, six trials with 6 and/or 10 metre deep plankton nets (350 µm mesh size) around fish cages were performed to test preventive effects on sea lice transmission. In five of the trials, a few cages per site were covered with 6 and/or 10 meter deep nets. In the sixth trial, all cages in one location were covered by 10 meter deep nets. Environmental and health-related variables, such as oxygen, salinity, mortality and disease outbreaks, were observed during the study. Associations between the use of plankton nets and infestations of sea lice were examined by regression analysis. The results show that sea lice infestations in cages shielded by either 6 or 10 meters deep plankton nets were significantly reduced. The largest effect was seen with use of 10 meter deep plankton nets. In these field trials, no significant effects on oxygen levels or negative health conditions were observed. It is recommended however, to ensure adequate monitoring of oxygen levels as the oxygen conditions may be different at different farm locations. We conclude that the use of plankton nets significantly reduces sea lice transmission to farming cages, and that the use of plankton nets as a continuous barrier against infectious copepodites is an effective new tool to control sea lice.

Lumpfish: Optimizing the use of a new cleaner fish

A. Johannesen[1]* and R. Arge[1]

[1]*Fiskaaling, Hvalvik, Faroe Islands*

Cleaner fish have been used in the Atlantic Salmon farming industry for over a decade with mixed results. The fish commonly used are various species of wrasse; most recently ballan wrasse (*Labrus bergylta*). However, wrasse are not native to some of the farming regions in the North Atlantic and for environmental reasons, importing non-native species is not recommended. As an alternative, lumpfish (*Cyclopterus lumpus*) have in recent years been used in Norway and work is currently ongoing in Norway, Iceland and the Faroe Islands to breed and test the cleaning efficiency of lumpfish. While it is clear that lumpfish do show interest in consuming salmon lice (*Lepeophtheirus salmonis*), breeding practices have not been optimised (*e.g.* captive broodstock have not been established) and indications so far are that only approximately one third of lumpfish are inclined to clean. In order to optimise the use of lumpfish as cleaner fish, it is necessary to implement better breeding and rearing practices as well as understanding the behaviour of lumpfish and what might motivate cleaning behaviour. Combining knowledge of lumpfish personality and good broodstock selection should increase the proportion of cleaning lumpfish. We present some initial results from breeding, rearing and behavioural studies. Early results indicate 1) a need to use live feed in the first few weeks post hatching, 2) surface area as opposed to volume is important for good welfare and growth, and 3) that lumpfish activity levels are connected with interest in salmon lice.

Delousing efficiency of farmed ballan wrasse (*Labrus bergylta*) against *Lepeophtheirus salmonis* infecting Atlantic salmon post-smolts

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[1]*Institute of Aquaculture, University of Stirling, Stirling, Scotland, UK.*

The use of cleaner fish against sea lice is rapidly emerging as a central component of Integrated Sea Lice Management (ISLM) in the European Atlantic salmon industry. To sustain this promising biological method in Scotland, ballan wrasse farming and the optimisation of their deployment at sea are currently supported by a joint industry-academic effort co-founded by the Technology Strategy Board (TSB, 2012-2014).

A series of comparative delousing trials performed in a tank-system will be presented. Each trial used Atlantic salmon post-smolts (~ 150 to 300 g), high initial lice infection levels (~ 5 to 12 lice / salmon) and farmed ballan wrasse naïve to previous exposure to sea-lice and salmon stocked at commercially relevant wrasse: salmon ratios (5 % to 8 %).

Sea-lice load declined following a one-phase exponential decay model at a rate of 0.8 to 1.3% / hour for each wrasse stocked per 100 salmon with levels below 0.5 lice / salmon typically obtained within 84 h. The wrasse body-mass and the availability of fresh opened blue mussels (*Mytilus edulis* L.) did not significantly affect delousing but severe jaw deformity did. The functional predator response was linear showing no minimum prey density threshold for sea lice foraging and no satiation plateau despite the high consumption rates measured. This series of trials confirmed farmed specimens as highly effective therapeutic and preventive agents against sea lice. The implications of the present findings will be summarised along with additional observations and future priorities.

Natural sea lice mitigation at an Atlantic salmon (*Salmo salar*) farm in British Columbia, Canada, using cultured filter-feeding bivalves

A. Byrne[1,2]*, C.M. Pearce[1,2,3], S.F. Cross[2,4], S.R.M. Jones[1,2] and S.M.C. Robinson[5,6]

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[2]*University of Victoria, Victoria, British Columbia, Canada*
[3]*Vancouver Island University, Nanaimo, British Columbia, Canada*
[4]*SEA Vision Group Inc., Courtenay, British Columbia, Canada*
[5]*St. Andrews Biological Station, Fisheries and Oceans Canada, St. Andrews, New Brunswick, Canada*
[6]*University of New Brunswick Saint John, Saint John, New Brunswick, Canada*

The close proximity of net-pen salmon farms and wild Pacific salmon stocks in the Broughton Archipelago region of British Columbia is an important incentive for precautionary sea lice management strategies. We are investigating the ability of filter-feeding bivalves to provide preventative, natural louse control through the ingestion of sea lice larvae (nauplii and copepodids) in the water column. Field trials are underway at a commercial Atlantic salmon (*Salmo salar*) farm in the Broughton Archipelago, where two species of sea lice – *Lepeophtheirus salmonis* and *Caligus clemensi* – are present. Pacific oysters (*Crassostrea gigas*), one of several bivalve species that consumed sea lice larvae in previous laboratory trials, have been deployed in trays at three depths around one end of the farm's 2 x 7 cage array, and at a nearby control site without fish. Shell measurements and tissue weights (wet, dry, ash-free dry) of bivalves around the farm have been significantly larger than those of control animals. Control and experimental oyster digestive tracts are preserved bi-monthly and will be analyzed for partially digested sea lice and louse DNA using microscopy and qPCR, respectively. Planktonic sea lice and those attached to salmon are counted monthly in treatment and control (without oysters) fish cages. This system exploits sea louse life history and the natural filtration capabilities of bivalves in an effort to reduce sea lice populations at the farm in an environmentally-responsible manner. Evidence of lice ingestion and/or population reduction by oysters would encourage further development and adoption of alternative, non-chemical louse control techniques.

Notes

Tuesday – Plenary Talk

Dr. Stephen Bocking

Professor of Environmental History and Policy, and Chair of the Environmental and Resource Science/Studies Program, at Trent University

Science, Salmon, and Sea Lice: Understanding a Persistent Debate

For nearly three decades scientists have been studying sea lice, salmon farms, and ecosystems. Much has been learned regarding the physiology and ecology of sea lice and their interactions with farmed and wild salmonids. And yet these interactions remain the focus of debate, both within and beyond the scientific community – contradicting the common assumption that, with sufficient research, it is possible to generate a consensus regarding scientific facts. I present an historical perspective on this debate, with the aim of understanding how and why it has persisted, even in the face of extensive research. My perspective includes an analysis of key episodes in the history of sea lice science, such as the research that developed after observation of sea lice on sea trout in Ireland and Scotland after 1990, and on pink salmon in British Columbia after 2001. I will suggest that this debate is rooted in the local environmental histories of sea lice and salmon farms, as well as in the complexities and challenges – both ecological and social – that are inherent to sea lice science. In addition, sea lice have persisted as the focus of debate because they often serve as surrogates for larger debates about the industry that cannot be resolved through scientific research.

Notes

Tuesday – Session 4
Epidemiology, Modeling and Analysis

Session Chairs:
Nabeil Salama
Damian Brady

Session Titles:

9:00 Towards an assessment of regional salmon lice infection pressure
L. Asplin*, P.A. Bjørn, I.A. Johnsen, A.D. Sandvik, J. Skardhamar, J. Albretsen, B. Ådlandsvik, M.S. Myksvoll, R. Nilsen, R. M. Serra-Llinares and U. Lindstrøm

9:15 The predictability of sea lice density in Norwegian fjords
Anne D. Sandvik*, Jofrid Skarðhamar, Pål-Arne Bjørn, Lars Asplin, Serra Rosa Maria Llinares and Ingrid A. Johnsen

9:30 Using a biologically assessed sea lice transport model to determine dispersal characteristics for informing management
N.K.G. Salama*, C.C. Pert, A.G. Murray, I.S. Wallace, J. Dunn, J.G. Fraser, B. Rabe and C.M. Collins

9:45 Modeling dispersion of Caligus rogercresseyi from 102 salmon farm sites in Chile using a 3D hydrodynamic model (SINMOD) and displaying the results in an innovative user-friendly interface
J.E. Unibazo*, Ø. Knutsen and D. Slagstad

10:00 Modelling the influence of wild salmon on the evolution of resistance to chemotherapeutants in sea lice (Lepeophtheirus salmonis)
G. F. McEwan*, M. L. Groner, C. W. Revie, M. D. Fast

10:15

10:30 Break sponsored by Maine Aquaculture Innovation Center

Towards an assessment of regional salmon lice infection pressure

L. Asplin[1]*, P.A. Bjørn[1], I.A. Johnsen[1], A.D. Sandvik[1], J. Skardhamar[1], J. Albretsen[1], B. Ådlandsvik[1], M.S. Myksvoll[1], R. Nilsen[1], R. M. Serra-Llinares[1] and U. Lindstrøm[1]

[1]*Institute of Marine Research, Bergen, Norway*

Salmon lice are regarded as a threat to the wild salmonid population along the Norwegian coast. Particularly after the establishment of salmon aquaculture, the last ~30 years has seen an increase in the number of hosts for the parasite by a factor of several hundreds. In order to monitor the wild fish population strength and the potential harm caused by salmon lice, we need to be able to quantify the infection pressure. In the last 5-10 years the Institute of Marine Research has conducted an intensive monitoring of salmon lice copepodite abundance and distribution in several regions in Norway as a part of the Norwegian governmental salmon lice monitoring program. We use various field sampling techniques as well as different models in the surveillance. We will show the experiences of this huge monitoring program so far and we will describe the development towards an operational model based assesment for salmon lice infection pressure scheduled for 2015 and 2017.

The predictability of sea lice density in Norwegian fjords

Anne D. Sandvik[1]*, Jofrid Skarðhamar[2], Pål-Arne Bjørn[2], Lars Asplin[1], Serra Rosa Maria Llinares[2] and Ingrid A. Johnsen[1]

[1]*Institute of Marine Research, Bergen, Norway*
[2]*Institute of Marine Research, Tromsø, Norway*

The Norwegian aquaculture industry aims to be environmentally sustainable with no population regulating effects on wild fish. With high density of fish farms, and thus potential hosts for sea lice, the number of hatched sea lice eggs can at times be enormous in some areas. It addition it has been shown that the released nauplii can be transported over large distances before they reach the stage of infective copepodids. Thus, the density of infective sea lice copepodids can differ significantly from the density of released nauplii, and hence a sophisticated prediction/warning system is needed.

In the present work we have compared observations of sea lice counts on wild fish with model estimated sea lice densities in water masses. Data from a systematic monitoring program of sea lice are available from 2010 to present. For the same period model estimated sea lice densities have been made available through a combination of a three dimensional hydrodynamic model system (NorKyst-800m) and an individual-based sea lice tracing model, where the nauplius input data have been estimated based on sea lice counts, biomass and water temperature as reported from the fish farms.

The correlation between the two data sets will be presented and discussed, and examples will be given on how the model system can be used in a carrying capacity context.

Using a biologically assessed sea lice transport model to determine dispersal characteristics for informing management

N.K.G. Salama[1]*, C.C. Pert[1], A.G. Murray[1], I.S. Wallace[1], J. Dunn[1], J.G. Fraser[1], B. Rabe[1] and C.M. Collins[1]

[1]*Marine Scotland Science, Aberdeen, Scotland*

A hydrodynamic model describing one of Scotland's largest fjordic systems, coupled with a sea lice biological particle-tracking model, predict sea lice transport, distributions and farm connectivity during May and October 2011 – 2013. Sentinel cages and plankton trawls allowed an assessment of the simulations when weighted by transformed relative farm lice counts as an input source.

Results to date indicate that sea lice are distributed heterogeneously within the system, and the gradation in observations corresponds to the ranking of the simulated lice distributions, indicating that the model describes the general characteristics of sea lice transport within the system. Simulations from each farm result in 50% of lice being transported within 6km, >95% within 15km, with a small proportion simulated to disperse up to 35km. During the simulation periods, the number and strength of direct farm connectivity varies, with farms forming < five direct links, with the majority being unconnected to each other and eight being self-exposing. The probability of connectivity for sea lice exposure ranges of the order 10^{-5} – 10^{-2}.

The connectivity probabilities could provide parameters for developing system population models and inform co-operative region structures. Simulated distributions may inform on locations of proposed farms to minimise exposure. Although distance between farms does not necessarily equate to connection strength, the simulated transport distances provide support for disease management areas (developed for ISAV intervention) illustrating possible additional benefits for sea lice management. It may also be possible to use a measure of connectivity to inform efforts relating to targeted monitoring and intervention.

Modeling dispersion of *Caligus rogercresseyi* from 102 salmon farm sites in Chile using a 3D hydrodynamic model (SINMOD) and displaying the results in an innovative user-friendly interface

J.E. Unibazo[1]*, Ø. Knutsen[2] and D. Slagstad[2]

[1]*AVS Chile S.A., Puerto Varas, Chile.*
[2]*SINTEF Fisheries and Aquaculture, Trondheim, Norway.*

The interior sea of central Chiloé Island (Chile) is highly populated by salmon farms comprising around 150 farming sites. *Caligus rogercresseyi* causes large economic losses to the salmon producers mainly due to increased susceptibility to secondary infections and treatment costs. Thus it is important for the sustainability of the industry to understand how *Caligus* behave in the water column and in the currents, considering its biological characteristics, in order to favour sound decisions on managing farming areas in terms of treatment co-ordination, stocking and harvesting. In this project, we simulated the dispersion of *Caligus* and virus-like particles using a high resolution hydrodynamic 3D model, SINMOD. While particles representing both *Caligus* and viruses were assumed to flow freely with the currents, the model further included a behavioural component for *Caligus*, as they display vertical swimming behavior modulated by depth and salinity. After all particle tracks were calculated in SINMOD, we used the relative fraction of particles from one location that hit another location (102 farm sites, 9 producer companies). The results show the percentage of infection transfer between sites, which were represented in an innovative and user-friendly web interface where the salmon producers can simulate several infection scenarios (Figure 1). We concluded that oceanographic modeling can improve knowledge of how *Caligus* and viruses behave in the water column, as well as understanding the interaction between farm sites. This, combined with the novel user-friendly interface for results display, should favour sound management decision-making.

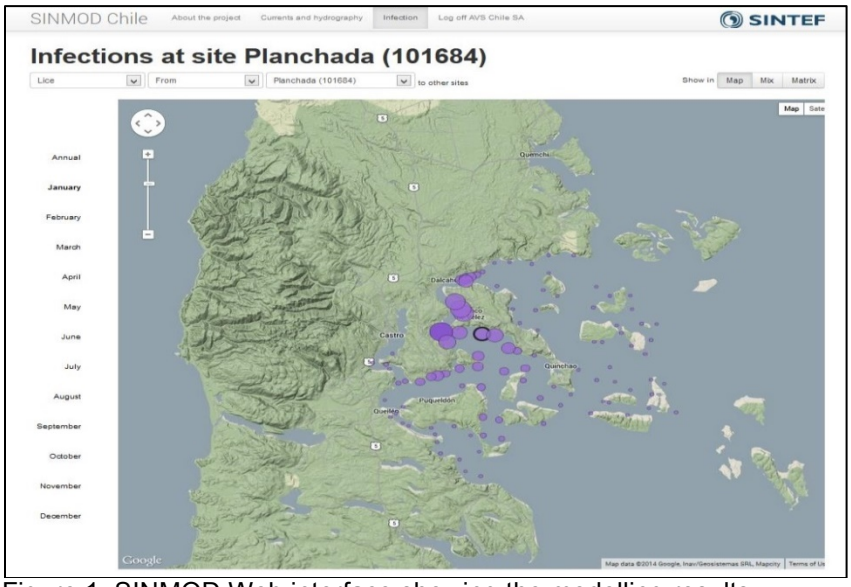

Figure 1: SINMOD Web interface showing the modelling results

Modeling the influence of wild salmon on the evolution of resistance to chemotherapeutants in sea lice (*Lepeophtheirus salmonis*)

G. F. McEwan[1]*, M. L. Groner[1], C. W. Revie[1], M. D. Fast[1]

[1]*University of Prince Edward Island (UPEI), Charlottetown, PE, Canada*

A growing problem for salmon farms is resistance to chemical treatments in sea lice. While some farming regions have severe resistance problems, others are relatively unaffected. The reasons for this variation are not clearly understood, resulting in *ad hoc* resistance management strategies. Understanding factors influencing evolution of resistance is important to formulate strategies that slow such evolution and increase the effective lifetime of treatments.

In some farmed animals, parasite resistance to chemical treatments is managed by leaving refugia during each treatment. Migration of parasites between the treated population and untreated refugia dilutes the genetic contribution of resistant individuals. This strategy is especially effective when there is a cost to resistance leading to selection for susceptible individuals in the refugia.

We propose that resistance to chemical treatments of sea lice on farms is reduced by exposure to wild salmon. Lice on wild salmonids are not exposed to treatments, and their offspring may infest farmed salmon. We hypothesise that wild salmonids act as refugia and hence farms with greater exposure to wild salmonids will evolve resistance more slowly.

We built an Agent Based Model of salmon farms including salmon hosts, sea lice parasites, chemical treatments, a resistant gene, and exposure to wild salmonids. We explored the evolution of treatment resistance under different scenarios of exposure to wild salmonids and resistance costs.

We demonstrate that farms with greater exposure to wild salmonid populations have slower evolution of resistance and require fewer treatments. Additionally, costs of resistance increase the influence of wild salmonid refugia.

Notes

Notes

Tuesday – Session 5
Sea Louse Biology I

Session Chairs:
Stewart Johnson
Rasmus Skern-Mauritzen

Session Titles:

11:00 The salmon louse life cycle: How did two chalimus stages become four chalimus stages?
C. Eichner, L.A. Hamre*, R. Skern-Mauritzen and F. Nilsen

11:15 Use of RAD sequencing to isolate a sex-specific SNP marker in the salmon louse *Lepeophtheirus salmonis* (Krøyer, 1837)
S. N. Carmichael, M. Bekaert, J. B.Taggart, J.H. Ireland, H. R. L. Christie, D. I. Bassett, J. E. Bron, P. J. Skuce, K. Gharbi, R. Skern-Mauritzen, A. Sturm*

11:30 Characterization of sex determination genes in salmon louse, Lepeophtheirus salmonis
M. Furne, C. M. A. Caipang, R. Skern-Mauritzen

11:45 Assessing the use of ATP as a condition index in the sea louse, Lepeophtheirus salmonis, in the Bay of Fundy
S.M.C. Robinson*, T.R. Lander, K.P. Ang

12:00 Nuclear receptors in salmon lice, Lepeophtheirus salmonis
R. Male*, M. Dondrup, I. Tolås, M. Khatri, K. Gravdal, P. Battachan, F. Nilsen

12:15 Characterization of salmon louse Lepeophtheirus salmonis, genes containing fibronectin type II domains
E. Harasimczuk, F. Nilsen, A. C. Øvergård, S. Grotmol, H. Kongshaug, S. Dalvin

12:30 Lunch sponsored by AquaPharma

The salmon louse life cycle: How did two chalimus stages become four chalimus stages?

C. Eichner[x1], L.A. Hamre[x1]*, R. Skern-Mauritzen[2] and F. Nilsen[1]

[x] *authors contributing equally.*
[1] *SLRC-Sea Lice Research Center, Department of Biology, University of Bergen* [2] *Aquatic pathogens and diseases, Institute of Marine Research, Bergen, Norway,*

Until recently a total of 10 stages in the life cycle of *Lepeophtheirus salmonis* was accepted and described morphologically in detail based on successive samples from host populations. In 2009 Ohtsuka *et al*. suggested that *L. salmonis* have two and not four chalimus stages in the life cycle as originally described. This was proven to be correct by observing and measuring chalimi molting in incubators (Hamre et al. 2013). Material from this study was analyzed focusing on growth during the chalimus phase of *L. salmonis*. A significant instar growth of about 35% was observed in both chalimus 1 and chalimus 2, equal among males and females. Cepalothorax increased by about 12% and posterior body by about 80%. Relative total length increase at molting was at the same order of magnitude as instar growth; however, molt growth was mainly constituted by increase of cepalothorax while instar growth was mainly due to an increase of the posterior body. At molting females increased significantly more than males in length. Sexual size dimorphism was established upon molting to the chalimus 2 stage in which cepalothorax length displayed a clear bimodal distribution. Duration of each chalimus stage was one day shorter for males than females. The former misinterpretation including four and not two chalimus stages in the life cycle of *L. salmonis* was caused by significant instar growth. This was difficult to detect in a series of population samples, particularly when accompanied by sexual size dimorphism and differential development rate of male and female chalimi that could not be sex determined at the time.

References

L.A. Hamre, C. Eichner, C.M.A. Caipang, S.T. Dalvin, J.E. Bron, F. Nilsen, G.A. Boxshall, R. Skern-Mauritzen, 2013. The salmon louse *Lepeophtheirus salmonis* (Copepoda: Caligidae) life cycle has only two chalimus stages. Plos One 8.

S. Ohtsuka, I.Takami, B.A.V. Maran, K. Ogawa, T. Shimono, Y. Fujita, M. Asakawa, G.A. Boxshall, 2009. Developmental stages and growth of *Pseudocaligus fugu* Yamaguti, 1936 (Copepoda: Siphonostomatoida: Caligidae) host-specific to Puffer. J Nat Hist 43, 1779-1804.

Use of RAD sequencing to isolate a sex-specific SNP marker in the salmon louse *Lepeophtheirus salmonis* (Krøyer, 1837)

S. N. Carmichael[1], M. Bekaert[1], J. B.Taggart[1], J.H. Ireland[1], H. R. L. Christie[1], D. I. Bassett[1], J. E. Bron[1], P. J. Skuce[3], K. Gharbi[3], R. Skern-Mauritzen[4], A. Sturm[1]*

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The salmon louse, *Lepeophtheirus salmonis* (Copepoda, Crustacea), is a fish parasite causing significant economic damage to the commercial production of Atlantic salmon (*Salmo salar*). While sex ratios in the gonochoristic species *L. salmonis* are usually close to 1:1, its mechanism of sex determination is unknown. This study provides evidence for a genetic mechanism of sex determination in *L. salmonis*, based on the identification of a sex-specific Single Nucleotide Polymorphism (SNP) marker. Restriction site-associated DNA sequencing (RAD-seq) was used to isolate a large number of SNP markers and achieve the parallel genotyping of male and female individuals of a laboratory-maintained *L. salmonis* strain. From a total of 85 million raw sequence reads, 281,838 unique RAD markers were obtained. RAD marker Lsa101901 showed complete association with sex for all individuals analysed, being heterozygous in females and homozygous in males. This SNP association pattern was further confirmed for two unrelated parasite strains using allele-specific PCR assays. Complete association with phenotypic sex was observed in a total of 96 genotyped individuals. The marker Lsa101901 mapped to the coding region of the prohibitin-2 gene. Prohibitin-2 mRNA levels determined by RT-qPCR were sex-dependent, with adult females showing about 1.8-fold higher transcript levels than adult males. In summary, results of this study provide evidence that sex determination in *L. salmonis* is genetic and follows a female heterozygous system. Marker Lsa101901 can be used to determine the genetic sex in *L. salmonis*, and could be useful in the development of control strategies.

Characterization of sex determination genes in salmon louse, *Lepeophtheirus salmonis*

M. Furne[1], C. M. A. Caipang[1], R. Skern-Mauritzen[1]

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Sex determination is a fundamental developmental process controlling gonad differentiation, sex-specific physiological development, morphology and behavior. Putative gene homologues to insect genes involved in the sex determination cascade (*sex lethal LsSxl*, *transformer-2 Lstra-2* and *double sex Lsdsx)* have been identified in the salmon lice genome. These genes have been cloned and their structure determined in order to create molecular phylogenies with homologous genes from other species. To analyze the developmental expression pattern of *LsSxl*, *Lstra-2* and *Lsdsx*, RT-qPCR was performed on different life stages of the salmon lice (eggs, nauplii, chalimus, copepodites, pre-adult males and females, and adult males and females). *Lsdsx*, *Lstra-2* and *LsSxl* showed variation in the expression along the life cycle. Whereas *Lstra2* and *LsSxl* are expressed in all life stages following a similar expression pattern, *Lsdsx* is mainly expressed in nauplii and is highly downregulated in copepodites, preadults and adults males and females. These results will be discussed along with results from RNAi experiments that indicate inter-regulatory relationships.

Assessing the use of ATP as a condition index in the sea louse, *Lepeophtheirus salmonis*, in the Bay of Fundy

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The problem of sea lice continues to plague the at-sea growing operations of Atlantic salmon (*Salmo salar*) causing significant annual economic losses. To control outbreaks of these parasites, several approaches have been used to control the attached juvenile/adult stages such as: in-feed pharmaceutical prescriptions, chemo-therapeutant bath treatments, predators and various farm management techniques such as fallowing.

Since the reproductive output of sea lice can be quite high in outbreak conditions, our research has been investigating the early life history of the sea louse, *Lepeoptheirus salmonis*, with an emphasis on the larval stages. In this particular study, we examined the ontogenetic changes in adenosine triphosphate (ATP) levels in post-hatch larval sea lice over a two week period. ATP is the ubiquitous biochemical energy source in all organisms. Results showed a negative response in ATP over time which correlated well with a decrease in the volume of the internal oil droplet contained in the body of the larvae. This decrease in ATP was accentuated in a parallel group of larvae from the same cohort that was stimulated to swim more actively with a light stimulus.

These results suggest that the ATP concentration in a sea louse larva, measured with a simple assay, is related to its age and its remaining energy stores. This observation may be useful as a condition index of larvae captured in the field for ecological-based studies. Future research will use this technique to measure the relative attachment efficiency (infection pressure) of larvae on salmon in controlled conditions.

Nuclear receptors in salmon lice, *Lepeophtheirus salmonis*

R. Male[1]*, M. Dondrup[1], I. Tolås[1], M. Khatri[1], K. Gravdal[1], P. Battachan[1], F. Nilsen[1]

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Nuclear receptors (NRs) are ligand activated transcription factors that share common structural organization and are found to be conserved from nematodes to mammals. They are defined by a double zinc-finger type DNA binding domain, a characteristic ligand binding domain and a variable linker between these conserved regions. In addition NRs have a highly variable N-terminal (A/B region) and an often unstructured C-terminal region. Sea lice have approximately 25 different nuclear receptors, about the same number as in insects, and half the number of receptors found in mammals. All receptors have a well-defined DNA binding domain of around 90 amino acids and a ligand binding domain, more variable but still clearly conserved. The N-terminal A/B domain is highly variable ranging from a few residues to more than 400 amino acids (like in HR38). Some NR genes show alternative splicing that will give changes in this region. The C-terminal (F domain) varies from a few to more than 500 amino acids. The structure of ligand binding domains suggests potential for binding of small molecules in many receptors, but only the ecdysone receptor (EcR) has been shown to do so at nM range. However, other receptors may be activated by ligands at higher concentration. Other receptors like HR38 have bulky residues in the ligand binding cleft that probably discriminate against ligand binding. The gene structures of nuclear receptors in salmon lice are highly variable, but some evolutionarily conserved patterns of introns are noted and will be discussed.

Characterization of salmon louse *Lepeophtheirus salmonis,* genes containing fibronectin type II domains

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Salmon louse, *Lepeoptheirus salmonis* (Krøyer), is an obligate ecto-parasite feeding on mucus, skin and blood. The parasite occurs on most species in the genera *Oncorhynchus, Salmo and Salvelinus* in the Northern hemisphere. Treatments against salmon lice are limited to a few pesticides, and development of resistance towards these has challenged lice management in aquaculture. New treatment methods are thus needed to ensure a sustainable industrial production of salmon.

Fibronectin (FN) is a multi-domain glycoprotein found in both plasma and extracellular matrix of vertebrates, playing an important role in cell differentiation, cell adhesion and wound healing. Fibronectin contains three distinct functional domains, which are called fibronectin type I, II, and III. The type II domain has until recently been considered vertebrate specific, since invertebrates usually possess the putative ancestral kringle domain. Genome searches have revealed that the salmon louse possesses approximately 75 genes with variable numbers of FN II domains. Some of these genes have architectures that include a peptidase domain.

Sequences of several genes that contain FN II domains were obtained by 5`and 3`RACE. Gene transcription levels at all developmental stages throughout the lifecycle have been obtained using qPCR, while *in situ* hybridization has been performed to reveal transcription sites. Moreover, the functional role has been studied by RNA interference.

Notes

Tuesday – Session 6
Sea Louse Biology II

Session Chairs:
Ben Koop
Sussie Dalvin

Session Titles:

1:30 Digestion and reproduction are inhibited by RNA interference mediated knockdown of LsKDELR and LsCOPB2 in the salmon louse
C. Trösße*, F. Nilsen and S. Dalvin

1:45 Identification of an intracellular cystatin in L. salmonis subsp. salmonis and its putative role the digestive process and immune responses
C.M.A. Caipang, S. Mæhle, E.P. Garcia and R. Skern-Mauritzen*

2:00 Molecular characterisation of the ecdysone receptor (EcR) in the salmon louse, Lepeophtheirus salmonis
L. Sandlund* , F. Nilsen, R. Male, H. Kongshaug, S. Grotmol and S. Dalvin

2:15 Identification and characterisation of Halloween genes in sea lice: investigating a novel group of drug targets
C.M. McNair*, J.H. Ireland, Q. Zhong, S.J. Monaghan and J.E. Bron

2:30 Molecular characterization of a salmon louse (Lepeophtheirus salmonis) chitinase using RNA interference in planktonic stages
C. Eichner, E. Harasimczuk, S. Grotmol, F. Nilsen, S. Dalvin*

2:45 The ABC gene family of the salmon louse (*Lepeophtheirus salmonis*)
S.N. Carmichael, J. Heumann, J.E. Bron, M. Bekaert and A. Sturm*

3:00 Recent advances in the production and implementation of farmed ballan wrasse (*Labrus bergylta*) in the Scottish salmon industry
E. Leclercq, B. Grant, A. Chalaris, A. Davie and H. Migaud*

3:15 Break sponsored by Merck Animal Health

Digestion and reproduction are inhibited by RNA interference mediated knockdown of LsKDELR and LsCOPB2 in the salmon louse

C. Trösße[1]*, F. Nilsen[2] and S. Dalvin[3]

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Knockdown of genes by RNA interference is a valuable tool to investigate the functions of the respective gene products and to screen for possible targets for pest management employing chemicals or vaccines. Two secretory pathway components involved in the retrograde transport of proteins from the endoplasmic reticulum to the Golgi have been identified and knocked down in the salmon louse (*Lepeophtheirus salmonis*): LsKDELR, a receptor recognising and sorting endoplasmic reticulum resident proteins; and LsCOPB2, a subunit of the protein coat that covers retrograde transport vesicles. qRT-PCR analysis revealed that both genes were highly expressed in nauplii and adult females. *LsKDELR* and *LsCOPB2* localisation in adult females was determined by *in situ* hybridisation. Both transcripts were found in the ovaries and the oocytes; and LsCOPB2 was also found in the gut. *LsKDELR* and *LsCOPB2* were knocked down by RNA interference in preadult females. Knockdown animals were sampled at the adult stage, when the control group had extruded at least the second pair of egg strings. The knockdown was confirmed by qRT-PCR. *LsCOPB2* knockdown lice had a significantly higher mortality and failed to develop normally, while both *LsCOPB2* and *LsKDELR* knockdown caused disturbed digestion and the absence of egg strings. The findings presented here show the potential of LsKDELR and LsCOPB2 as suitable target candidates for new pest control methods.

Identification of an intracellular cystatin in *L. salmonis* subsp. *salmonis* and its putative role the digestive process and immune responses

C.M.A. Caipang[1], S. Mæhle[2], E.P. Garcia[2] and R. Skern-Mauritzen[2]*

[1]*Temasek Polytechnic, Singapore, Singapore*
[2]*Institute of Marine Research, Bergen, Norway*

Cystatins are a superfamily of proteins that specifically inhibit cysteine proteases. An intracellular type-1 cystatin, *Lscystatin-1* has been identified from salmon louse, *Lepeophtheirus salmonis* subsp. *salmonis*. *Lscystatin-1* is a type-1 intracellular cystatin with homologs identified in ticks and it is closely related to a type-1 cystatin of *Caligus rogercresseyi*. The gene is ubiquitously expressed in salmon louse, with high expression in the egg stage as well as in the pre-adult and adult stages and low expression in the nauplii, copepodid and chalimus stages. Its expression is enhanced by exposure of the adult lice to immunostimulants including lipopolysaccharide (LPS) and poly I:C and also induced in fully fed lice. Incubation of salmon lice eggstrings with a transfection reagent containing *Lscystatin-1* dsRNA resulted in almost complete silencing, while partial inhibition of transcription was observed in *Lscystatin-1* dsRNA-exposed nauplii and in the injected adult lice. There was low recovery of both *Lscystatin-1* dsRNA-injected lice and the control lice from the experimentally infected fish. However, the relative reduction rate of the *Lscystatin* dsRNA-injected lice from fish was moderate (> 50%). These results collectively suggest that cystatin is involved in both the digestive processes and immune response in salmon lice.

Molecular characterisation of the ecdysone receptor (EcR) in the salmon louse, *Lepeophtheirus salmonis*

L. Sandlund* [1], F. Nilsen[1], R. Male[2], H. Kongshaug[1], S. Grotmol[1] and S. Dalvin[3]

[1]*Sea Lice Research Centre, University of Bergen, Department of Biology Bergen, Norway*
[2]*Sea Lice Research Centre, University of Bergen, Department of Molecular Biology, Bergen, Norway*
[3]*Sea Lice Research Centre, Institute of Marine Research, Bergen, Norway*

The salmon louse *Lepeophtheirus salmonis* (Copepoda, Caligidae) is an important parasite in the salmon farming industry in the Northern Hemisphere causing annual losses of hundreds of millions US dollars. In order to facilitate development of a vaccine or other novel measures to gain control of the parasite, knowledge of molecular biological functions of *L. salmonis* is vital. In arthropods, developmental processes, such as reproduction and oogenesis, are mediated by binding of steroid hormones to a heterodimer comprised of the ecdysone receptor (EcR) and a homolog of the retinoid X receptor, ultraspiracle (USP). In this study, full-length cDNA of the *L.salmonis* EcR (*Ls*EcR) was obtained by 5`RACE PCR and characterized. *Ls*EcR amino acid sequences have high sequence identities in the DNA binding and ligand binding domain to other arthropod EcRs, including *Tribolium castaneum* and *Tigriopus japonicus*. Moreover, *in situ* analysis of adult female louse revealed *LsEcR* transcript to be localized in a variety of tissues such as ovaries, sub-cuticular tissue and oocytes. Furthermore, the functional role of *LsEcR* was investigated using RNA interference.

Identification and characterisation of Halloween genes in sea lice: investigating a novel group of drug targets

C.M. McNair[1]*, J.H. Ireland[1], Q. Zhong[1], S.J. Monaghan[1] and J.E. Bron

[1]*Institute of Aquaculture, University of Stirling, Stirling, United Kingdom*

In recent years it has become increasingly evident that the current control methods for the salmon louse, *Lepeophtheirus salmonis*, are failing. Resistance to emamectin benzoate (SLICE®) has been observed in lice populations[1], and it is clear that alternative drug targets and/or alternative control methods are necessary. A potential drug target in arthropods such as *L. salmonis* is the moulting process. Drugs such as chitin synthesis inhibitors are available for control of sea lice, *e.g*. diflubenzuron[2]; however, moulting hormone itself has not been targeted as yet. Moulting hormone is an ecdysteroid, making it a difficult target, but the cytochrome P450 enzymes which process active moulting hormone (20-hydroxyecdysone) could offer novel targets for drugs and vaccines.

In arthropods, moulting hormone is synthesised from cholesterol taken up in the diet through a series of enzymatic reactions. Each of these reactions is driven by a cytochrome P450 enzyme. These enzymes are encoded by a gene family termed the Halloween genes. Homologues of two Halloween genes, *disembodied* and *shade,* were identified in *L. salmonis* ESTs, and full length sequences were amplified using RACE PCR. Expression of these genes was analysed in different life-cycle stages of *L. salmonis*, from egg through to adult. These genes will be knocked down using RNA interference to investigate whether or not they are essential to the moulting process, which will help to ascertain whether they will make useful targets for novel drugs and vaccines for the future control of salmon lice infestations.

References:

[1]S.N. Carmichael, J.E. Bron, J.B. Taggart, J.H. Ireland, M. Bekaert, S.T. Burgess, P.J. Skuce, A.J. Nisbet, K. Gharbi and A. Sturm. Salmon lice (*Lepeophtheirus salmonis*) showing varying emamectin benzoate susceptibilities differ in neuronal acetylcholine receptor and GABA-gated chloride channel mRNA expression. *BMC Genomics* 14(1):408.

[2]T.E. Horsberg and T. Høy 1991. Tissue distribution of 14C-diflubenzuron in Atlantic salmon (*Salmo salar*). *Acta. Vet. Scand.* 32(4): 527-533.

Molecular characterization of a salmon louse (*Lepeophtheirus salmonis*) chitinase using RNA interference in planktonic stages

C. Eichner[1], E. Harasimczuk[2], S. Grotmol[1], F. Nilsen[1], S. Dalvin[2]*

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[2]*Sea Lice Research Centre, Institute of Marine Research, Bergen, Norway*

Chitin is a structural polysaccharide found in the cell walls of fungi and the exoskeletons of most arthropods and marine invertebrates. In insects, the chitinases are present mainly in the moulting fluid and midgut. In blood-eating insects such as mosquitoes, chitin is also found in the peritrophic membrane that is formed in the midgut after ingestion of blood. The ability to digest food and to undergo cycles of molt are essential functions for the salmon louse and hence genes involved in these processes offers opportunities as potential vaccine targets. The main goal of this study was therefor to investigate function and expression of a salmon louse chitinase named LsChi2 (*Lepeophtheirus salmonis* chitinase 2). Here, we present a molecular characterization of LsChi2 including phylogeny, sequence analysis and expression patterns. Furthermore, functional data for LsChi2 was obtained by performance of RNA interference in planktonic stages to monitor molting performance. Subsequently, LsChi2 knock–down copepodites were also tested for their ability to infect fish.

The ABC gene family of the salmon louse (*Lepeophtheirus salmonis*)

S.N. Carmichael[1], J. Heumann[1], J.E. Bron[1], M. Bekaert[1] and A. Sturm[1]*

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The large ABC (ATP-binding cassette) gene family contains membrane proteins mediating the trafficking of inorganic ions, metals, sugars, amino acids, peptides, lipids and drugs. The enhanced expression of ABC drug transporters can confer chemical resistance to cancers, pathogens and pests. The ABC transporter P-glycoprotein has been proposed as a factor contributing to emamectin benzoate resistance in the salmon louse (*Lepeophtheirus salmonis*) [1, 2]. Here, we report a systematic survey of the ABC gene family in *L. salmonis* based on searches of an extensive transcriptome generated by Illumina HiSeq 2000 paired-end sequencing of a multi-stage mRNA library of the parasite (RNA-Seq). TopHat and Cufflinks were used to assemble 389,927,940 raw sequence reads, using the *L. salmonis* genome as a reference [http://sealouse.imr.no/]. Reads that could not be aligned by this strategy were assembled *de novo*. The resulting assembly of 33,933 transcripts comprised 30,159 unique and 3,774 alternatively spliced transcripts. The *L. salmonis* transcripts, as well as 128,783 available ESTs [3], were scanned for ABC transporters using Hidden Markow Models (HMM) for protein patterns unique to the gene family. Using this strategy, 39 *L. salmonis* ABC genes were identified of which 20 were represented both in the transcriptome and ESTs, while 13 and 6 were unique to the transcriptome and EST pool, respectively. A significant number of the identified genes were affiliated to subfamilies known to contain drug transporters such as ABCB (3 genes), ABCC (15 genes) and ABCG (7 genes). This catalogue of *L. salmonis* ABC genes will support further toxicological studies.

References

[1] N.D. Tribble, J.F. Burka, and F.S.B. Kibenge. 2007. Evidence for changes in the transcription levels of two putative P-glycoprotein genes in sea lice (*Lepeophtheirus salmonis*) in response to emamectin benzoate exposure. Mol. Biochem. Parasitol. 153:59–65.

[2] J. Heumann, S.N. Carmichael, J.E. Bron, A. Tildesley and A. Sturm. 2012. Molecular cloning and characterisation of a novel P-glycoprotein in the salmon louse *Lepeophtheirus salmonis*. Comp. Biochem. Physiol. Pt. C 155: 198–205.

[3] R.Yazawa, M. Yasuike, J. Leong, K.R. von Schalburg, G.A. Cooper, M. Beetz-Sargent, A. Robb, W.S. Davidson, S.R.M. Jones and B.F. Koop. 2008. EST and mitochondrial DNA sequences support a distinct Pacific form of salmon louse, *Lepeophtheirus salmonis*. *Mar. Biotechnol*. 10: 741–9.

Recent advances in the production and implementation of farmed ballan wrasse (*Labrus bergylta*) in the Scottish salmon industry

E. Leclercq[1], B. Grant[1], A. Chalaris[1], A. Davie[1] and H. Migaud[1]*

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The use of cleaner fish against sea lice is rapidly emerging as a central component of Integrated Sea Lice Management (ISLM) in the European Atlantic salmon industry. To sustain this promising biological method in Scotland, ballan wrasse farming and the optimisation of their deployment at sea are under intense development with the benefit of a strong industry-academic partnership.

The poster presentation will summarise key partners, activities, findings and commercial milestones achieved since 2010 with emphases on the output of the project “Production and Implementation of farmed ballan wrasse (*Labrus bergylta*) in the Scottish salmon industry”. This project (2012-2014) is co-founded by the Technology Strategy Boards and the two leading Scottish salmon farmers: Marine Harvest and Scottish Sea Farms and supports the activities of a dedicated ballan wrasse hatchery (Machrihanish Marine Farm; Machrihanish, UK). Additional work within the second Scottish ballan wrasse hatchery (Otter Ferry Seafish, UK) will also be summarised in addition to recent efforts to establish and consolidate best commercial practices for the management of cleaner-fish upon deployment at sea (Sainsbury`s R&D Agricultural Research Fund, 2014-2015).

Notes

Notes

Tuesday – Session 7
Sea Louse Biology III

Session Chairs:
Sandra Bravo
Ben Sutherland

Session Titles:

3:45 LiceBase: Model organism database and functional genomics tools for the sea lice research community
M. Dondrup*, C. Andreetta, I. Jonassen, F. Nilsen

4:00 Sublethal threshold of Caligus rogercresseyi (Boxshall & Bravo 2000) on the physiological response of the host Salmo salar (Linnaeus 1758)
M.P. González*, L. Vargas-Chacoff and S.L. Marín

4:15 Caligus rogercresseyi transcriptome: Novel insights for key biological processes during the lifecycle of the salmon louse
C. Gallardo-Escárate, V. Valenzuela-Muñoz, G. Núñez-Acuña, J. Chávez-Mardones, W. Maldonado-Aguayo, A. T. Gonçalves, R. Farlora, D. Valenzuela-Miranda

4:30 Microarray profiling in skin revealed protective mechanisms mediated by feeding plant derived anti-lice bioactives against salmon lice in Atlantic salmon
H. Holm, S. Wadsworth, A.K. Osmo, A. Krasnov, Ø. Evensen, S.Skugor*

4:45 Development of bacterial 'microbiome-markers' for salmon microbiota mediated resistance against infection with sea louse, Lepeophtheirus salmonis
S. Leadbeater*, N. Derome, M. Llewellyn, K. P. Ang, F. Powell and J. Elliot

5:00 Detection and quantification of planktonic Lepeophtheirus salmonis by real-time PCR
A. Mols-Mortensen*, G. á Norði, E. Danielsen, Á. Jacobsen, D.H. Christiansen and R. Skern

LiceBase: Model organism database and functional genomics tools for the sea lice research community

M. Dondrup[1]*, C. Andreetta[1], I. Jonassen[1], F. Nilsen[1].

[1]*Sea lice Research Centre, University of Bergen, Bergen, Norway*

High-throughput sequencing, functional genomics and bioinformatics are playing an increasingly important role in sea lice research. As a prominent example, the genome sequence of the Atlantic salmon louse *Lepeophtheirus salmonis* has been sequenced recently. A cornerstone of the search for novel drug- or vaccine targets is the correct functional annotation of the genome. We present LiceBase as a database to leverage the wealth of emerging data on the sea louse genome and to aid the design of novel experiments.

LiceBase is a web-based system for storing, visualizing, and data-mining which built on the GMOD-tools (http://gmod.org) that also drive major model organism databases such as FlyBase or WormBase. These model organism databases have had a large impact on the annotation process and have become established tools for their respective research communities.

High-throughput sequencing of the transcriptome (RNA-seq) of *L. salmonis* at all life-cycle stages and various tissues has recently been performed, and the analysis results are now available for visualization through our genome browser. We have also developed a Laboratory Information Management System (LIMS) to annotate RNA-interference experiments and to associate phenotypes and genotypes of gene knock-down experiments. LiceBase further provides Blast searches, InterProScan, and GO annotation. LiceBase is available at https://licebase.org and will be open to the community upon publication of the genome.

Sublethal threshold of *Caligus rogercresseyi* (Boxshall & Bravo 2000) on the physiological response of the host *Salmo salar* (Linnaeus 1758)

M.P. González[1]*, L. Vargas-Chacoff[2] and S.L. Marín[3]

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Physiological effects produced by the ectoparasite *Caligus rogercresseyi* on *Salmo salar* cultures in Chile are scarce. The establishment of an infestation intensity threshold, above which sub-lethal physiological effects on the host occurs is relevant to the improvement of management strategies. The aim of this study was to quantify the effects of parasite abundance of attached and mobile stages on the physiological response of *S. salar*. Fish (n=100) were infected with different abundances of copepodids and 50 remained uninfected. Cortisol, glucose, proteins, amino acids, triglycerides, lactate and osmolality concentrations in plasma, and number and diameter of skin mucous cells were quantified. Sampling was performed at time 0 (before infection), and 1, 8, 16 and 22 days post-infection. Simple and piecewise regressions were used to determine the relationships between physiological variables and parasite abundance, and to identify sub-lethal threshold parasite intensity (break points), respectively. A decrease in plasma concentrations with increasing parasite loads were detected for proteins, amino acids, triglycerides, mucous cell diameter (with an increase in cell number) and osmolality under chalimus I-II; glucose and proteins under chalimus III-IV; and osmolality under adults. Break points were detected for fish infected with adult parasites. The values were 6-7 for glucose and cortisol; 14 for mucous cell number and diameter; and 20-21 for proteins, amino acids, triglycerides and lactate. *C. rogercresseyi* causes a load-dependent effect, increasing stress response and energy demand with high loads. These results suggest that break point of 14-15 lice should be considered in the context of threshold for applying parasite treatments.

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Caligus rogercresseyi transcriptome: Novel insights for key biological processes during the lifecycle of the salmon louse

C. Gallardo-Escárate[1], V. Valenzuela-Muñoz[1], G. Núñez-Acuña[1], J. Chávez-Mardones[1], W. Maldonado-Aguayo[1], A. T. Gonçalves[1], R. Farlora[1], D. Valenzuela-Miranda[1]

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Despite the economic and environmental impacts that sea lice infestations have on salmon farming worldwide, genomic data generated by high-throughput transcriptome sequencing for different developmental stages, sexes, and strains of sea lice is still limited or unknown. In this study, RNA-seq analysis was performed using *de novo* transcriptome assembly as a reference for evidenced transcriptional changes from six developmental stages of the salmon louse *Caligus rogercresseyi*. EST-datasets were generated from the nauplius I, nauplius II, copepodid and chalimus stages and from female and male adults using MiSeq Illumina sequencing. A total of 151,788,682 transcripts were yielded, which were assembled into 83,444 high quality contigs and subsequently annotated into roughly 24,000 genes based on known proteins. To identify differential transcription patterns among salmon louse stages, cluster analyses were performed using normalized gene expression values. Herein, four clusters were differentially expressed between nauplius I-II and copepodid stages (604 transcripts), five clusters between copepodid and chalimus stages (2,426 transcripts), and six clusters between female and male adults (2,478 transcripts). Gene ontology analysis revealed that the nauplius I-II, copepodid and chalimus stages were mainly annotated to amino acid transfer/repair/breakdown, metabolism, molting cycle, and nervous system development. Additionally, genes showing differential transcription in female and male adults were highly related to cytoskeletal and contractile elements, reproduction, cell development, morphogenesis, and transcription-translation processes. The data presented in this study provides the most comprehensive transcriptome resource available for *C. rogercresseyi*, which should be used for future genomic studies linked to host-parasite interactions.

Microarray profiling in skin revealed protective mechanisms mediated by feeding plant derived anti-lice bioactives against salmon lice in Atlantic salmon

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[3]*Nofima, Ås, Norway*

Repelling parasites from attaching by masking the smell of the host or by interfering with the attachment process could play an important role in integrated pest management strategies on Atlantic salmon farms. We hypothesized that dietary exposure to anti-lice compounds would exert a protective effect against lice. Several trials were performed and specific plant derived anti-lice bioactives will be discussed.

Three groups of Atlantic salmon were fed control diet and two test diets enriched with anti-lice bioactive compounds at low and high dose. Pre-challenge feeding period lasted for 3 weeks and continued throughout the lice-challenge for 21 more days. Highest protection was achieved by the low dose diet – 28% reduction in lice counts compared to control. Skin samples taken from behind the dorsal fin were analyzed by the 21k oligonucleotide microarray and qPCR to study gene expression responses in close proximity to the sites of lice attachment.

To study the effects of diets prior to lice infection, uninfected fish under the high dose were compared to control. Remarkably, we revealed an activation of pro-inflammatory skin responses; especially interesting was the induction of 44 interferon related genes. Lice infected fish exposed to anti-lice bioactives also had higher expression of these and numerous other immune genes compared to fish fed control diet. On the other side, higher lice counts on control fish were strongly associated with the up-regulation of genes involved in secretory pathways. In conclusion, dietary modulation of skin tissue responses may be an effective way to protect fish against lice.

Development of bacterial 'microbiome-markers' for salmon microbiota mediated resistance against infection with sea louse, *Lepeophtheirus salmonis*

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At the Saint Andrews Biological Station (Department of Fisheries and Oceans Canada) collaborative research activities have been undertaken to better understand sea lice (*Lepeophtheirus salmonis*) biology, their relationship to their environment and fish and the treatments and methods used to reduce their abundance near aquaculture rearing sites. Of these, improving our understanding of inherent family related differences in resistance to sea lice holds promise to reduce treatment frequency and improve fish health. A research project with Cooke Aquaculture and Laval University is focused on finding differences in the microbial community that may lead to tools that can be applied to future offspring. The microbiota is substantially involved in a wide range of host functions, including immune defense. This close interaction has been suggested to result from a highly co-evolved symbiosis influenced by nutrition, physiology and immunological factors. This Aquaculture Collaborative Research Development Program (ACRDP) project represents a starting point in understanding the potential role of salmon skin and gut microbiological ecology in protection against parasites and pathogens. 1200 salmon representing 40 families were exposed to copepodid stage sea lice, which were allowed to settle on the salmon. Mucus, fecal samples and lice counts were collected over one month to the near completion of the lice life cycle. *In-vivo* lab trial logistics, differences in lice load as well as preliminary differences in microbiota composition will be presented.

Detection and quantification of planktonic *Lepeophtheirus salmonis* by real-time PCR

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Sea lice (Copepoda, Caligidae) have a large economic impact on salmonid aquaculture, and have since the dawn of the Atlantic salmon (*Salmo salar*; Linnaeus, 1758) fish farming industry. Sea lice have been a widespread pathogenic marine parasite with escalating infestation on other cultured fish as well as wild salmonids. In the Faroe Islands *Lepeophtheirus salmonis* (Krøyer, 1837) is the most dominant pathogenic parasite, and the industry spends significant resources fighting the infection of *L. salmonis* and keeping within the legislation limits. The planktonic stages of *L. salmonis* include two non-feeding nauplius stages and an infective free-living copepodid stage. Little is known about behavior and distribution of the planktonic stages even though this is considered crucial in developing farming patterns that may reduce infection risk. Sampling, identifying and counting the planktonic stages of sea lice using traditional methods including morphological identification and microscopy is a bottle-neck to gaining better insights into the behavior and distribution of the sea lice. The approach is highly time consuming and requires trained and skilled personnel. McBeath *et al*. (2006) developed a molecular method to detect *L. salmonis* that utilized real-time PCR targeting of the mitochondrial gene cytochrome oxidase subunit I (COI). The method was developed to enable discrimination between *L. salmonis* and *Caligus elongates*, and the potential of the method to serve as a quantitative measure of planktonic *L. salmonis* was also explored. Here we focus on developing a quantitative method to estimate the concentration of planktonic *L. salmonis* in the environment using real-time PCR.

Reference:

A.J.A. McBeath, M.J. Penston, M. Snow, P.F. Cook, I.R. Bricknell & C.O. Cunningham 2006. Development and application of real-time PCR for specific detection of *Lepeophtheirus salmonis* and *Caligus elongates* larvae in Scottish plankton samples. *Dis. Aqua. Org.* 73: 141-150

Notes

Thursday – Plenary Talk

In memory of Peter Andreas Heuch and Rod Wootten

Karin Kroon Boxaspen

Institute of Marine Research, Bergen Norway

The development of sea lice biology research over 30years – from salmon farm production problems to environmental impact issues and state-of-the-art research

Two outstanding scientists with large contributions to the field of sea lice research have passed away since our last Sea Lice conference in 2012. I have had the pleasure of working with Peter Andreas Heuch (PA) from the start of our scientific careers and knew Rod Wootten over the same period through contact at many conferences over the years, where his calm presence and great knowledge was most appreciated.

The field of sea lice research has, in this period, developed its focus from one where sea lice were of concern only as a production problem on salmon farms to the situation today where sea lice are also recognised as an environmental issue. In the latest risk assessment of the environmental impact of Norwegian Atlantic salmon farming, sea lice are classified as one of the two most important issues, with escapees being the second.

Sea louse research, which initially concentrated on basic biology, treatments and basic infection parameters, largely assessed by individuals and local research groups, now involves internationally collaborative, multidisciplinary science employing a range of state-of-the-art techniques. Sea lice research now includes a wide selection of novel methods and approaches in biology, immunology, epidemiology, genetics, genomics and hydrodynamic modelling to mention only a few areas.

A historic overview of the development of this research field, including both PA's and Rod's highly significant contributions, show us how far we can get with a combination of good science and co-operation.

Notes

Thursday – Session 8
Use of Chemotherapeutants, Modes of Action, Targets, and Resistance I

Session Sponsored by: Merck Animal Health

Session Chairs:
John Burka
Armin Sturm

Session Titles:

9:00 Screening of pharmaceutical compounds for effect on preadult salmon lice
S.M. Aaen* and T.E. Horsberg

9:15 Are laboratory bioassays an efficient tool for monitoring Caligus rogercresseyi (Boxshall & Bravo 2000) sensitivity to antiparasitics: Weaknesses and strengths
S.L. Marín*, R. Ibarra, M.H. Medina

9:30 Trends in the success of pyrethroid and organophosphate bath treatments against Caligus rogercresseyi in the Chilean salmon industry
A. Tello*, P. Artacho, R. Ibarra and M. H. Medina

9:45 First report of hydrogen peroxide resistance in salmon lice (Lepeophtheirus salmonis) in Norway
K.O. Helgesen*, H. Romstad, S.M. Aaen and T.E. Horsberg

10:00 Emamectin benzoate field data from Norway from spring 2011 until spring 2014
K. Ulven*, B. Lygren

10:15 Break

Screening of pharmaceutical compounds for effect on preadult salmon lice

S.M. Aaen[1]* and T.E. Horsberg[1]

[1]*Norwegian University of Life Sciences, Sea Lice Research Centre, Oslo, Norway*

Currently, five classes of medicinal compounds are available for removal of sea lice from cultivated salmonids. These are avermectins, flubenzurones, hydrogen peroxide, organophosphates and pyrethroids. A growing number of fish farming sites are experiencing reduced sensitivity towards one or more of these compounds, a trend that severely impacts the sustainability of the industry. Thus, novel medicinal products are needed.

In a series of experiments, preadult salmon lice from a strain sensitive to existing treatment agents were exposed to 25 compounds with different modes of action according to the IRAC classification (http://www.irac-online.org/teams/mode-of-action/). The initial concentration was 50 mg L^{-1} for all compounds, which were dissolved in sea water by using a 50/50 solution of emulsion and dimethyl sulfoxide. The exposure time was 30 minutes. After keeping the parasites in sea water for 24 hours, the mortality was recorded. Compounds resulting in 100 % mortality were further investigated by exposing parasites to declining concentrations until the lowest concentration was found. The following compounds were included in the latter exposure:

Group	**Compound**
Fenylpyrazol	Pyriprole
Neonicotinoid	Imidacloprid
Nereistoxin analogue	Cartap
Pyrazin-isoquinoline derivate	Praziquantel

Some compounds proved effective to some extent at 50 mg mL^{-1}. These results indicate that several mechanisms can be utilized as targets in the preadult stages of salmon lice.

Are laboratory bioassays an efficient tool for monitoring *Caligus rogercresseyi* (Boxshall & Bravo 2000) sensitivity to antiparasitics: Weaknesses and strengths

S.L. Marín[1]*, R. Ibarra[2], M.H. Medina[2]

[1]*Instituto de Acuicultura, Universidad Austral de Chile, Puerto Montt, Chile*
[2]*Instituto Tecnológico del Salmón, INTESAL de SalmonChile, Puerto Montt, Chile*

The variety of antiparasitics against Caligid copepods is limited and efforts to maintain their efficacy are needed. Bioassays are used to monitor parasite responses to these chemotherapeutants, but its usefulness for making decisions over the short term regarding applying a given product, need to be analyzed. The objective of this study was to evaluate weaknesses and strengths of bioassays as a tool for monitoring *Caligus rogercresseyi* response to antiparasitics based on deltamethrin, cypermethrin, azamethiphos and emamectin benzoate. Bioassay design consisted of exposing parasites collected from a farm to the different chemoterapeutants at the authorized concentration and exposure times, under laboratory conditions and evaluating the percentage of dead parasites 48 h post exposure. Parasite sampling design consisted in collecting parasites from 8 farms across Los Lagos and Aysén regions (Southern Chile) and performing the bioassays one farm at a time. This procedure was repeated 3 times. Among weaknesses, significant variability in the actual antiparasitic concentrations reported from the laboratory must be highlighted, together with the high spatial and temporal variability of parasite responses. While the former may affect results interpretation, the latter indicates that parasite responses may be highly dynamic, making short term decisions regarding what antiparasitic use in a given farm very unpredictable. However, the large spatial and temporal scale considered in this study allowed detection of trends in areas where parasites seemed to be more sensitive to the tested chemotherapeutants. Thus, bioassay strengths may be found when they are used for detecting both large spatial and temporal trends.

This study was funded by Instituto Tecnologico del Salmon, INTESAL de SalmonChile. The authors acknowledge the collaboration given by some pharmaceutical and salmon producers who facilitated the performance of the study at different stages.

Trends in the success of pyrethroid and organophosphate bath treatments against Caligus rogercresseyi in the Chilean salmon industry

A. Tello[1]*, P. Artacho[1], R. Ibarra[1] and M. H. Medina[1]

[1]*Instituto Tecnológico del Salmón, INTESAL de SalmonChile, Puerto Montt, Chile*

Infection by the sea lice species' *Caligus rogercresseyi* is a key sanitary challenge in the Chilean salmon industry. Currently, infections are primarily controlled by the use of therapeutic bath treatments using the pyrethroid insecticides cypermethrin and deltamethrin and, as of 2013, the organophosphate azamethiphos. In this study we use the largest weekly sea lice abundance dataset of the Chilean salmon industry to characterize spatial and temporal trends in the treatment success of cypermethrin, deltamethrin and azamethiphos. We analyzed sea lice abundance data at the pen, farm site, management zone and industry level to describe treatment success variability at multiple spatial and temporal scales. Our results show a considerable degree of variation in treatment success at the spatial and temporal scales analyzed and differing trends in the treatment success of the different compounds. Importantly, they strongly suggest that, in many cases, sea lice sensitivity is not the major factor driving bath treatment performance. Overall, our results highlight the relevance of promoting data-driven bath treatment strategies to optimize the use of available chemicals and promote best management practices.

First report of hydrogen peroxide resistance in salmon lice (*Lepeophtheirus salmonis*) in Norway

K.O. Helgesen*[1], H. Romstad[2], S.M. Aaen[1] and T.E. Horsberg[1]

[1]*Norwegian University of Life Sciences, Sea Lice Research Centre, Oslo, Norway*
[2]*Aqua kompetanse AS, Flatanger, Norway*

Resistance towards chemotherapeutants in the salmon louse *Lepeophtheirus salmonis* is an increasing problem in the Norwegian fish farming industry. Most fish farmers are dependent on chemical treatments in order to keep the salmon lice count below authority required levels. As resistance emerges this task becomes increasingly difficult. Hydrogen peroxide is an agent used for delousing in bath treatments. No treatment failures caused by resistant parasites have, until recently, been documented from Norway. The aim of the current study was to develop a bioassay protocol suitable for hydrogen peroxide sensitivity testing on *L. salmonis,* and to use this protocol to find the sensitivity level of parasites. Furthermore the objective was to investigate whether a failed treatment was caused by parasite resistance, and if so; whether this reduced sensitivity was heritable.

The study was performed on a sensitive lab strain with no previous history of exposure to hydrogen peroxide and on six strains of salmon lice from the northern part of Mid-Norway previously treated with hydrogen peroxide. One of the latter strains was from a farm with several episodes of reduced treatment efficacy. The bioassay protocol was based on the pyrethroid bioassay protocol described in Sevatdal and Horsberg, 2003, with some modifications.

The results show that it is possible to distinguish between strains of salmon lice with regards to sensitivity towards hydrogen peroxide, using bioassays. The study also revealed reduced sensitivity in parasites from a farm with reduced treatment efficacy, both in the F0- and the F1-generation.

References:

S. Sevatdal & T.E. Horsberg (2003). Determination of reduced sensitivity in sea lice (*Lepeophtheirus salmonis* (Krøyer)) against the pyrethroid deltamethrin using bioassays and probit modelling *Aquaculture* 218(1-4):21-31

Emamectin benzoate field data from Norway from spring 2011 until spring 2014

K. Ulven*[1], B. Lygren[1]

[1]*MSD Animal Health, Bergen, Norway*

Emamectin benzoate (EB) has been one of the major active ingredients used to control salmon lice (*Lepeophtheirus salmonis)* in farmed Atlantic salmon (*Salmo salar*) in Norwegian aquaculture since 2000. Up until 2009 the other main chemical compounds in use were deltamethrin and cypermethrin. In 2009 diflubenzuron, teflubenzuron and azamethiphos were also re-introduced into the sea lice treatment tool box. Additionally, hydrogen peroxide has been used during the last few years to alternate between different chemical compounds.

An Integrated Pest Management (IPM) model has been used by the aquaculture industry with the intention of delaying the onset of resistance by sea lice to the available medicines. Despite this, reduced sensitivity has been demonstrated against most of the treatments in use.

It has been a field approach, via the SLICE® Sustainability Program (SSP), to position the use of EB in an IPM context, including monitoring of susceptibility patterns of sea lice to EB. From spring 2011 fish farmers were encouraged to share bioassay data prior to treatment to demonstrate salmon lice resistance levels, to submit skin/muscle samples following treatment to indicate EB concentrations in the fish and to share salmon lice clearance data after treatment.

This paper will give a descriptive presentation of the data collected from spring 2011 to spring 2014. Results demonstrate the importance of bioassay surveillance for improved treatment efficacy and as a basis for more sustainable product rotation strategies. After introduction of the SSP the EB concentrations in fish improved compared to historical data prior to this program.

Notes

Notes

Thursday – Session 9
Use of Chemotherapeutants, Modes of Action, Targets, and Resistance II

Session Sponsored by: Fish Vet Group

Session Chairs:
Mark Fast
Sean Monaghan

Session Titles:

10:45 Avermectin treatment for Lepeophtheirus salmonis and effects on salmon immunophysiology
M.D. Fast*, K.E. Fitzpatrick, S.L. Purcell, S.C. Johnson, S. Wadsworth, S.K. Whyte

11:00 Emamectin benzoate resistant Salmon lice (Lepeophtheirus salmonis) show changes in ligand-gated ion channel expression
S.N. Carmichael, J.E. Bron, J.B. Taggart, J.H. Ireland, M. Bekaert, S.T.G. Burgess, P.J. Skuce, A.J. Nisbet, K. Gharbi & A. Sturm*

11:15 Transcriptomics of emamectin benzoate responses in resistant Atlantic and sensitive Pacific salmon lice Lepeophtheirus salmonis
B.J.G. Sutherland*, J.D. Poley, O.O. Igboeli, J.R. Jantzen, M.D. Fast, B.F. Koop, S.R.M. Jones

11:30 Global gene expression analysis of copepodid sea lice (Lepeophtheirus salmonis) drug responses using in vitro bioassays
J.D. Poley*, B.J. Sutherland, S.K. Whyte, O.O.Igboeli, S.L. Purcell, K.E. Fitzpatrick, B.F. Koop, M.D. Fast

11:45 Identification of the mechanism behind resistance against organophosphate (azamethiphos) in salmon lice (Lepeophtheirus salmonis)
KP. Kaur*, K. O. Helgesen, M. J. Bakke, T. E. Horsberg

12:00 Azamethiphos resistance – Frequency of resistance alleles in Norwegian salmon
KP. Kaur, S.M. Aaen, K. O. Helgesen, V. Aspehaug, T. E. Horsberg*

12:15 Lunch sponsored by EWOS Innovation

Avermectin treatment for *Lepeophtheirus salmonis* and effects on salmon immunophysiology

M.D. Fast[1]*, K.E. Fitzpatrick[1], S.L. Purcell[1], S.C. Johnson[2], S. Wadsworth[3], S.K. Whyte[1]

[1]*Atlantic Veterinary College, University of Prince Edward Island, Charlottetown, Prince Edward Island, Canada*
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[3]*EWOS Innovation Inc., Dirdal, Norway*

Throughout the 1990s the in-feed treatment, SLICE^{TM} (Emamectin benzoate-EMB) was so successful against *Lepeophtheirus salmonis*, that it was used almost exclusively in the Canadian aquaculture industry, providing lasting protection against all life stages of the parasite. Recently this treatment has exhibited reduced efficacy due to the development of resistance by sea lice in Eastern Canada, Chile, UK and Norway. Reduced efficacy has resulted in increased dosages of EMB (double and triple dosages, *etc*.) being used within the Bay of Fundy as well as the returned usage of ivermectin, a macrocyclic lactone related to EMB, as an off-label, in-feed chemotherapeutant. Ivermectin has demonstrated significant efficacy, however there are serious issues relating to toxicity. We investigated the effects of different EMB dosages, and multiple ivermectin treatments on baseline immunophysiolocial indicators, anti-viral responses and protection against subsequent sea lice exposure in salmon smolts. Different doses of EMB or repeated treatment with ivermectin did not affect feeding behaviour in salmon, however by the end of the second ivermectin treatment some neurotoxicity was observed. Single dose EMB (50 ug/kg) was ineffective at significantly impacting *L. salmonis* infection, whereas triple dosages (150 ug/kg) significantly reduced lice development and eliminated subsequent stress responses in salmon associated with lice development to pre-adult stages. Immunological gene regulation will also be discussed with respect to these findings.

Emamectin benzoate resistant Salmon lice (*Lepeophtheirus salmonis*) show changes in ligand-gated ion channel expression

S.N. Carmichael[1], J.E. Bron[1], J.B. Taggart[1], J.H. Ireland[1], M. Bekaert[1], S.T.G. Burgess[2], P.J. Skuce[2], A.J. Nisbet[2], K. Gharbi[3] & A. Sturm[1]*

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[2]*Moredun Research Institute, Penicuik, United Kingdom*
[3]*The GenePool, Edinburgh, United Kingdom*

The avermectin emamectin benzoate (EMB) is a component of the anti-sea louse drug SLICE ® (MSD Animal Health). Reports of decreased efficiency of SLICE suggest the development of EMB resistance in sea lice at least locally [1, 2]. Here, transcriptional profiling of salmon louse (*Lepeophtheirus salmonis*) laboratory strains differing in EMB susceptibility was used in order to identify potential EMB resistance factors. *L. salmonis* expressed sequence tags (EST) [3], as well as sequences obtained by suppression subtractive hybridisation between EMB-resistant (R) and susceptible (S) strains of the parasite, were assembled to give contiguous sequences and annotated. Based on these resources, 15K oligonucleotide microarrays were made. In the absence of drug exposure, 359 microarray targets were differentially expressed between the S and R strains, and these genes were enriched for functions such as calcium binding, chitin metabolism and muscle structure. Subunits of g-aminobutyric acid-gated chloride channels (GABA-Cl) and neuronal acetylcholine receptors (nAChR) showed significantly lower mRNA levels in R compared to S lice, with ~1.4-fold and ~2.8-fold changes, respectively, determined by RT-qPCR. R salmon lice showed few transcriptional responses following acute exposure to 200 µg L^{-1} EMB, a level toxic for S but not R lice. Avermectins exert their toxicity in arthropods through interaction with glutamate-gated chloride channels; however, further potential drug targets include GABA-Cl and nAChR. We hypothesise that the apparent down-regulation of GABA-Cl and nAChR subunits in EMB-hyposensitive salmon lice could be linked to the susceptibility phenotype, and may reflect roles of these receptors as avermectin target sites.

References:

[1] S. Bravo, S. Sevatdal, and T.E. Horsberg. 2010. Sensitivity assessment in the progeny of *Caligus rogercresseyi* to emamectin benzoate. *Bull. Europ. Assoc. Fish Pathol*. 30: 99–105.

[2] F. Lees, M. Baillie, G. Gettinby, and C.W. Revie. 2008. The efficacy of emamectin benzoate against infestations of *Lepeophtheirus salmonis* on farmed Atlantic salmon (*Salmo salar* L) in Scotland, 2002-2006. *PloS One* 3:2.

[3] R.Yazawa, M. Yasuike, J. Leong, K.R. von Schalburg, G.A. Cooper, M. Beetz-Sargent, A. Robb, W.S. Davidson, S.R.M. Jones and B.F. Koop. 2008. EST and mitochondrial DNA sequences support a distinct Pacific form of salmon louse, *Lepeophtheirus salmonis*. *Mar. Biotechnol*. 10: 741–9.

Transcriptomics of emamectin benzoate responses in resistant Atlantic and sensitive Pacific salmon lice *Lepeophtheirus salmonis*

B.J.G. Sutherland*[1], J.D. Poley[2], O.O. Igboeli[2], J.R. Jantzen[1], M.D. Fast[2], B.F. Koop[1], S.R.M. Jones[3]

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[3]*Pacific Biological Station, Nanaimo, British Columbia, Canada*

Transcriptomic approaches are expanding our understanding of systemic and local responses of salmon to sea lice infections, and of the genes and pathways involved in sea lice responses to environmental stressors. Recent emergence of resistance to the in-feed parasiticide emamectin benzoate (EMB; trade name SLICE^{TM}) and other important louse control drugs has prompted research into identifying mechanisms underlying resistance. Once identified, gene expression markers can improve resistance monitoring and our understanding of the selective pressures driving resistance development. Populations of Atlantic lice from multiple regions are now resistant to EMB, but Pacific lice in Western Canada remain sensitive. In this work we compare the transcriptomic responses of Pacific and Atlantic lice to EMB bioassays (Pacific: 0, 10, 25, 50 parts per billion (ppb); Atlantic: 0, 0.1, 25, 300, 1000 ppb). Atlantic lice were also separated by population (either high or low resistance) and sex; previous work has identified sex-specific differences in EMB sensitivity. In all cases, lice down-regulated degradative enzyme gene expression at higher doses of EMB (both Pacific and Atlantic lice). Although induced transcriptional responses were relatively moderate (*i.e*. fold change or number of differentially expressed genes), baseline differences between populations were large. Consistent in both sexes was the overexpression of peptidase transcripts in the highly resistant population. These results and several individual EMB response genes will be discussed and related to other drug resistant organisms.

Global gene expression analysis of copepodid sea lice (*Lepeophtheirus salmonis*) drug responses using *in vitro* bioassays

J.D. Poley[1]*, B.J. Sutherland[2], S.K. Whyte[1], O.O.Igboeli[1], S.L. Purcell[1], K.E. Fitzpatrick[1], B.F. Koop[2], M.D. Fast[1]

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[2]*Centre for Biomedical Research, Department of Biology, University of Victoria, Victoria BC, V8W 3N5, Canada*

The ectoparasitic copepod *Lepeophtheirus salmonis* affects wild and farmed salmonids in the Northern hemisphere, particularly in Norway, the United Kingdom and Canada. Through feeding on mucus, skin, and blood of the host, sea lice cause severe skin lesions, stress and mortality, imposing a financial burden of approximately $0.5 billion per year on salmonid aquaculture. Few treatment options are available and consequently, the industry has reported drug resistance to nearly all of the available compounds. The present study used microarray analysis to examine transcriptomic responses of free-swimming, infective sea lice copepodids when exposed to emamectin benzoate, azamethiphos, cypermethrin, or deltamethrin. Copepodid sea lice were exposed to a low and high dose of each drug *in vitro* for 24 hrs before assessing survival and collection for transcriptomic analysis using a 38K oligonucleotide microarray. Global transcriptomic responses analyzed by differential expression and principal components analysis indicated a limited induced transcriptional response to emamectin benzoate relative to other chemical treatments. Alternatively, deltamethrin and cypermethrin induced similar transcriptional responses, characterized by an enrichment of digestive enzymes and protease activity amongst others. Azamethiphos exposure caused an upregulation of several genes responsible for neural pathway regulation and also showed genes potentially involved in responding to organic compound exposure. The present study offers the first analysis of copepodid sea lice responses to several major chemotherapeutics used in aquaculture.

Identification of the mechanism behind resistance against organophosphate (azamethiphos) in salmon lice (*Lepeophtheirus salmonis*)

KP. Kaur[1]*, K. O. Helgesen[1], M. J. Bakke[1], T. E. Horsberg[1]

[1]*Norwegian University of Life Sciences, Sea Lice Research Centre, Oslo, Norway*

Acetylcholinesterase (AChE) is a critical enzyme in the nervous system that regulates the level of neurotransmitter acetylcholine and terminates the nerve impulses. AChE has been studied widely among arthropods in relation to resistance against organophosphates (OPs).

OPs have been used against salmon lice (*Lepeophtheirus salmonis*), in Norwegian salmonid aquaculture since late 1970s. The frequent use of OPs in Norway against *Lepeophtheirus salmonis* infestations resulted in the development of resistance in *L. salmonis,* leading to a major economic loss afflicting the aquaculture industry. Understanding and unraveling the biochemical pathways underlying the resistance in *Lepeophtheirus salmonis* against OPs is, therefore, the need of the hour.

In the present study, full length cDNA sequences encoding two AChEs in *Lepeophtheirus salmonis* were identified and fully characterized. Interestingly, in contrast to most other arthropods, *Lepeophtheirus salmonis* has two different forms of *ace1* gene (*ace1a* and *ace1b*) with no orthologous of *ace2* gene.

The screening of the whole cDNA sequences of *ace1a* and *ace1b*, in 5 sensitive and 5 resistant sea lice samples, led to the identification of a mutation in *ace1a*. This mutation was located at a highly conserved position in the active site gorge of AChE. In addition, the screening of various sensitive and resistant strains of *Lepeophtheirus salmonis*, for this mutation indicated its strong association with azamethiphos resistance. Hence, the integrated results of biochemical assay, mutation screening and 3D modelling strongly indicate that *ace1a* is likely the primary target for azamethiphos and the identified mutation is responsible for conferring reduced sensitivity in *Lepeophtheirus salmonis* against azamethiphos.

Azamethiphos resistance – Frequency of resistance alleles in Norwegian salmon

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Resistance towards the organophosphate azamethiphos in salmon lice has recently been demonstrated to be caused by a novel point mutation in the gene coding for the enzyme acetylcholin esterase by the sea lice resistance group at the veterinary school in Oslo. The company PatoGen AS has developed a rapid, high through-put TaqMan assay for this mutation, capable of performing large-scale screenings. In connection with development of this assay, a number of salmon lice samples were submitted from salmon farming companies and local fish health services to PatoGen AS for analysis for this biomarker. These were either random samples of parasites from the farm, or samples that were pre-selected through bioassays. The analysis of the data showed a very good association between the number of surviving parasites in bioassays and the frequency of the resistance marker. The analyses also showed that the resistance marker was present all along the Norwegian coast, except from the northernmost part. The frequencies varied though between different regions. As the analyses do not require live parasites (as bioassays do), and can be conducted on any developmental stage of the parasite, it has the potential to be a valuable tool for identification and management of azamethiphos resistance in salmon lice.

Notes

Thursday – Session 10
Sea Lice in Wild and Farmed Fish Populations

Session Chairs:
Ian Bricknell
Frank Kane

Session Titles:

1:15 Public perceptions and framing of salmon louse issues in Norway, the U.K. and Canada
M. Solberg, S. Dalvin*

1:30 Where are all the sea lice? Searching the wild fish of Cobscook Bay
A. Jensen, M. Pietrak*, S. Barker, G. Zydlewski and I. Bricknell

1:45 Where are all the sea lice? A first glance at sentinel fish in Cobscook Bay
C. Frederick*, M. Pietrak, S. Barker, D. Brady and I. Bricknell

2:00 Identifying variations in the potential infestation pressure from sea lice on wild salmonids in a Scottish salmonid aquaculture region
C.C. Pert*, S.J. Middlemas, C.M. Collins, D. Baum and N.K.G. Salama

2:15 Seasonal changes in the abundance of planktonic *Lepeophtheirus salmonis* and *Caligus elongatus* in a fish farming region in the Faroe Islands
G. á Norði*, K. Eliasen, E. Danielsen, K. Simonsen

2:30 Biology and ecology of sea lice on wild and farmed salmonids in the Strait of Georgia and Johnstone Strait, British Columbia, Canada
S.C. Johnson*, C-E.M. Neville, M. Trudel, and S.R.M. Jones

2:45 Occurrence of sea lice (Copepoda: Caligidae) on marine fishes from Jaramijo, an area with potential for sea-cage aquaculture in Ecuador
F.N. Morales-Serna*, P. Loor-Andrade, V. Caña-Bozada, G.B. Mera-Loor, and E.J. Fajer-Ávila

3:00 Break sponsored by Fish Vet Group

3:30 Poster Session sponsored by AquaPharma

Public perceptions and framings of salmon louse in Norway, the U.K. and Canada

M. Solberg[1], S. Dalvin[2]*

[1]*Department of Anthropology, University of Bergen, Bergen, Norway*
[2]*Sea Lice Research Centre, Institute of Marine Research, Bergen, Norway*

Salmon louse (*Lepeophtheirus salmonis*) infestation causes major problems for aquaculture in Norway, the U.K. and Canada. It poses a serious threat to animal welfare, and is believed by some scientists to jeopardize wild populations of salmonids. The distribution of knowledge concerning salmon lice among the public and consumers of aquaculture products is unknown, but believed to be low. The commonly observed confusion in Norway between the salmon louse and the distinctively different monogenean parasite *Gyrodactylus salaris* offers an anecdotal example.

This study investigates information and knowledge about salmon lice that is available online to consumers with limited prior knowledge of the issue. The study was performed by simple, systematic World Wide Web searches using the search engine Google. We have investigated the information provided in these hits and analyzed the contents with regards to: 1) Which actors disseminate and fund these informational websites, 2) and how issues about salmon lice are framed.

We argue that because different framings of environmental problems afford different (sometimes incompatible) solutions, any integrated pest management system and policies concerning salmon lice needs to take into account the highly divergent social perceptions that exist about salmon lice. This requires a basic understanding of the different scenarios, perceptions of risk, future threats, possible solutions and moral logics that are imagined by various actors, and guide actions in this contested field.

Where are all the sea lice? Searching the wild fish of Cobscook Bay

A. Jensen[1], M. Pietrak[1]*, S. Barker[1], G. Zydlewski[1] and I. Bricknell[1]

[1]*University of Maine, Orono, Maine USA*

The nature and role of the interactions between sea lice (*Lepeoptheirus salmonis*) and wild and farmed salmon is an area of ongoing debate. However, several key aspects can be agreed upon; farmed salmon smolts enter net pens free from lice and lice infect wild fish in the absence of farmed salmon. Salmon farmers in Cobscook Bay, Maine utilize a 3-year production cycle and coordinate stocking with nearby Canadian bay management zones in order to allow entire bays to remain fallow for several months to a year. These practices appear to reduce the initial infectious pressure when farms are restocked, but lice do eventually infect the farms. In an effort to better understand the local ecology of *L. salmonis* in Cobscook Bay, wild fish populations were surveyed for two years by seine net, intertidal fyke nets, benthic trawls and pelagic trawls, from March to November. Over 34 different species of fish were captured, but threespine sticklebacks (*Gasterosteus aculeatus*), blackspotted sticklebacks (*G. wheatlandi*), winter flounder (*Pseudopleuronectes americanus*), and mummichogs (*Fundulus heteroclitus*) were among the most abundant. Of note, no salmonids were captured during this effort. Caligid copepods were found on 12 different species of fish, but they were all *Caligus elongatus*. Over 3,000 threespined sticklebacks were caught and examined for lice. The lack of finding any *L. salmonis* present on them suggests that they likely do not play a significant role as a reservoir host in Cobscook Bay. Also the lack of finding *L. salmonis* on any of the wild fish also suggests that fallowing can be an effective strategy for managing sea lice on salmon farms in Cobscook Bay.

Where are all the sea lice? A first glance at sentinel fish in Cobscook Bay

C. Frederick[1]*, M. Pietrak[1], S. Barker[1], D. Brady[1] and I. Bricknell[1]

[1]*University of Maine, Orono, Maine USA*

The nature and role of the interactions between sea lice (*Lepeoptheirus salmonis*) and wild and farmed salmon is an area of ongoing debate. Cobscook Bay represents one of the primary salmon farming regions in Maine for more than 20 years. The Dennys River feeds the inner Cobscook Bay and contains one of the endangered Atlantic salmon populations in Maine. Four sentinel cages have been placed throughout the bay to better understand the seasonal variability in sea lice infectious pressure. The cages have been stocked for 1 week a month since June 2013 except for January and March. Environmental data including temperature, salinity, current speed and direction and light intensity have been collected. Preliminary data for the first year shows a seasonal increase in infection intensity and prevalence starting in August with a peak in October and November. The observed intensity and prevalence of lice infections and environmental data will help to establish and model where and when sea lice, whether shed from wild or farmed fish, infect migrating or farmed salmonids in a near shore ecosystem. The knowledge gained will provide vital information, regarding the understanding of the infectious pressure of sea lice in the coastal zone, the role of wild fish as hosts for sea lice, and lice infection dynamics over an aquaculture production cycle. This will enable the evaluation and refinement of the success of industry driven collaborative IPM efforts and provide a scientific framework to inform lease-granting bodies and marine resource users of the actual sea lice risk factors associated with wild fish population to salmon farms, or *vice versa*.

Identifying variations in the potential infestation pressure from sea lice on wild salmonids in a Scottish salmonid aquaculture region.

C.C. Pert[1]*, S.J. Middlemas[1], C.M. Collins[1], D. Baum[2] and N.K.G. Salama[1]

[1]*Marine Scotland Science, Aberdeen, Scotland, UK.*
[2]*Lochaber Fisheries Trust, Fort William, Lochaber, Scotland, UK.*

As part of a multi-disciplinary study in Loch Linnhe, located on Scotland's west coast, sentinel cages were deployed during April/May and October 2011-2013 to provide a biological assessment of infestation pressure. The position of cages was informed from model simulation outputs conducted for 2008. In addition, tagged wild salmonids were tracked in the system. Combining this data with sentinel, and netting survey data may provide an insight into the variation in settlement experienced by migrating smolts at different locations within the system.

The mean (±1 S.D.) settlement at times during the spring smolt migration was: 2011; 0.279 (0.294), 2012; 0.074 (0.096), 2013; 0.353 (0.420) with differences between sites inferred from the coefficient of variation (CV): 0.372, 0.428 and 0.397 respectively. During the same periods mean settlements on wild sea trout were 13.98 (18.51), 0.11 (0.35) and 8.98 (15.40).

At the end of production cycles mean settlements were 6.196 (4.086) and 2.649 (1.507) with reduced CV (0.233 and 0.215) indicating that settlement was greater and more evenly spread within the system. Mean settlement 0.074 (0.096) at the end of the first production year was similar to the beginning of production.

In May 2012, no lice were reported from farms, suggesting that 21 – 27% of lice in Linnhe did not originate from farmed fish within the system when compared to settlement in 2011 and 2013.

Settlement varies by location and stage of the farm production cycle. The rapid migration of salmon smolt through the system would suggest that their risk of accumulating detrimental lice burdens resulting in physiological consequences are low. However, due to their prolonged residence within the system, sea trout could be at higher risk.

Seasonal changes in the abundance of planktonic *Lepeophtheirus salmonis* and *Caligus elongatus* in a fish farming region in the Faroe Islands

G. á Norði[1]*, K. Eliasen[1], E. Danielsen[1], K. Simonsen[1]

[1]*Aquaculture Research Station of the Faroes, Hvalvík, Faroe Islands*

Since the onset of the fish farming industry, sea lice have affected the industry to various degrees. In the Faroes, the farmed fish is infected by two species *Lepeophtheirus salmonis* and *Caligus elongatus.* However, *L. salmonis* causes more damage, and thus the timing of treatments are mostly targeted towards *L. salmonis*. In this study we investigate the spatial and temporal distribution of planktonic sea lice in a strait with 5 active fish farms with coordinated farming cycles. The study commenced in November 2013, at which time, the fish farms were at the end of the growing period. In the first period of the study, *C. elongatus* was the dominant planktonic species, and the preliminary results shows the species composition to change with season and farming activity.

Biology and ecology of sea lice on wild and farmed salmonids in the Strait of Georgia and Johnstone Strait, British Columbia, Canada

S.C. Johnson[1]*, C-E.M. Neville[1], M. Trudel[1], and S.R.M. Jones[1]

[1]*Pacific Biological Station, Fisheries and Oceans Canada, Nanaimo, B.C., Canada*

In British Columbia it is generally accepted that sea lice transfers occur between wild and farmed salmonids, and that these transfers occur in both directions. What remains poorly understood is the magnitude of these transfers and the risk that they pose to wild salmon. To assess this risk we need to understand: the migratory pathways of wild salmon; the duration of their residency in the vicinity of fish farms; the species composition and abundance of sea lice on wild salmon; and the relative magnitude of the contributions from wild (salmonid and non-salmonid) and farmed hosts to sea lice levels seen on wild fish. From 2010 to 2012 we conducted large-scale surveys of wild juvenile salmon in the Strait of Georgia and Johnstone Strait. In addition to information on juvenile salmon distribution and migratory pathways we also determined the prevalence, abundance and developmental stages of *Lepeophtheirus salmonis* and *Caligus clemensi* on all salmon and select non-salmonid species. These data ***along with sea lice data from salmon farms, is*** providing insight into the biology of these copepods, their distribution and movements within the environment, and their impacts on salmon. This presentation will provide an overview of this program focusing on some of the challenges faced when interpreting sea lice data obtained from a complex ecosystem that contains numerous host species of different stocks and different migratory behaviors.

Occurrence of sea lice (Copepoda: Caligidae) on marine fishes from Jaramijo, an area with potential for sea-cage aquaculture in Ecuador

F.N. Morales-Serna*[1,2], P. Loor-Andrade[2], V. Caña-Bozada[2], G.B. Mera-Loor[2], and E.J. Fajer-Ávila[1]

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[2]*Universidad Laica Eloy Alfaro de Manabí, Manta, Ecuador*

Farming of finfish in sea cages is gaining popularity worldwide. These systems are a suitable environment for the emergence, establishment and transmission of parasites or pathogens, such as sea lice (Copepoda: Caligidae) known to cause serious diseases and economic losses in finfish aquaculture. In coastal waters of Jaramijó, Ecuador, there are plans to culture spotted rose snapper (*Lutjanus guttatus*) and longfin yellowtail (*Seriola rivoliana*); however, the information about the occurrence of sea lice on fish from this country is scarce. To address this problem, a parasitological survey of economically important fish caught by artisanal fishermen has been performed over the last year. Preliminary results are herein presented. A total of 558 fish belonging to 60 species distributed in 50 genera, 25 families, and 9 orders were examined. One hundred fifty eight individuals of Caligidae, belonging to 17 species, were found. The most common sea louse species was *Caligus asperimanus* from *Lutjanus argentiventris*, *L. guttatus*, *Haemulon steindachneri*, and *Calamus brachysomus*. The information provided in this study will support future research concerning prevention or epidemiology of sea lice as a potential pathogen in Ecuador fish farming.

Notes

Notes

Friday – Session 11
Host Immune Responses and Sea Louse Immuno-modulation

Session Chairs:
Sarah Barker
Carol McNair

Session Titles:

8:00 Recombinant vaccine efficacy trials against infectious larval salmon lice stages following intraperitoneal immunisation of Atlantic salmon with 9 vaccine candidates
S.J. Monaghan, C.M. McNair, H.C. McDonald, J.H. Ireland, S. Hamilton, D. Knox, W. Roy, K.D. Thompson, A. Adams, R.H. Richards, P.D. Smith, D. Bassett, C. Matthew, A. Preston, F. Groves, S. Boyd, T. Kanellos, D. Asper, J.E. Bron

8:15 Unravelling the acquired immune response to larval sea lice infections: a serological approach
S.J. Monaghan*, C.M. McNair, C. Metochis, K.D. Thompson, A. Adams, H.C. McDonald, S. Hamilton, D. Knox, R. Richards, P.D. Smith, W. Roy, T. Kanellos, D. Asper, J.E. Bron

8:30 Secretory / excretory products of *Lepeophtheirus salmonis* regulate salmon leukocyte migration in vitro
J.L. Piesz*, I.R. Bricknell, Hernan Pizarro, S.E. Barker

8:45 Tissue models for studying host-parasite interactions with salmon lice *Lepeophtheirus salmonis* (Copepoda, Caligidae)
H.C. McDonald*, A.P. Shinn, K.D. Thompson, K.F. Muir, S.J. Monaghan, C.M. McNair, R.H. Richards, D.P. Knox, S. Hamilton, D. Asper, T. Kanellos, J.E. Bron

9:00 Characterization and knock-down of a putative prostaglandin E synthase found in *Lepeophtheirus salmonis*
C. Eichner*, A. Øvergård, F. Nilsen, S. Dalvin

9:15 Increased susceptibility to infectious salmon anemia virus (ISAv) in *Lepeophtheirus salmonis* – infected Atlantic salmon
S.E. Barker*, J. Covello, D. Bouchard, W. Wolters, S. Purcell, M. Fast, I.R. Bricknell

9:30 Transcriptomic evidence for host-specific feeding responses of *Lepeophtheirus salmonis*
L.M. Braden*, B.J.G. Sutherland, B.F. Koop, S.R.M. Jones

9:45 Profiling the effects of plant derived anti-lice bioactives on salmon louse and Atlantic salmon
S. Skugor*, H. Holm, A.K. Osmo, T. Utne, A. Krasnov, Ø. Evensen, S. Wadsworth

10:00 Development of a vaccine against sea lice
Yamila Carpio González, Claudia García Castillo, Juana Maria Lugo González, Jannel Acosta Alba, Liliana Basabe Tuero, Antonio Morales, Reynold Morales, Osmany Rodrigo, Fidel Herrera, Janet Velazquez, Alexis Machin, Yeny Leal, Mario Pablo Estrada*

10:15 Break

Recombinant vaccine efficacy trials against infectious larval salmon lice stages following intraperitoneal immunisation of Atlantic salmon with 9 vaccine candidates

S.J. Monaghan[1]*, C.M. McNair[1], H.C. McDonald[1], J.H. Ireland[1], S. Hamilton[2], D. Knox[2], W. Roy[3], K.D. Thompson[1], A. Adams[1], R.H. Richards[1], P.D. Smith[4], D. Bassett[3], C. Matthew[3], A. Preston[3], F. Groves[3], S. Boyd[3], T. Kanellos[5], D. Asper[6], J.E. Bron[1]

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[2]*Moredun Research Institute, Midlothian, Edinburgh, UK*
[3]*Marine Environmental Research Laboratory, Machrihanish, UK*
[4]*Tethys Aquaculture, Ambo, Saffron Walden, Essex, UK*
[5]*Pfizer Inc. (France), 75668 Paris Cedex 14, France*
[6]*Zoetis Aquaculture Biologics Research, Kalamazoo, MI, United States*

The salmon louse, *Lepeophtheirus salmonis,* still comprises a barrier to sustainable economic growth in the Atlantic salmon aquaculture industry. As a result of acknowledged development of resistance by the parasite to a range of medicines, current research focuses on more environmentally friendly and cost-effective approaches to sea lice management[2,3]. A number of recombinant vaccines containing antigens expressed via *E. coli* or baculovirus expression systems (trypsin (Trypsin-VP2 and TM-L Tryp0), two mussel adhesion proteins (MAP-VP2 and TM-MAP#2), enolase, cystatin, vitellogenin and secretory/excretory products (SEPs) were assessed over two trials for their ability to reduce larval (chalimus) sea lice numbers following i.p. vaccination and challenge. After 600-700 degree days (dd) post-immunisation and 140 dd post-challenge, fish were sampled, with numbers of lice counted and blood collected. Specific serum antibody responses to the antigens were analysed by enzyme-linked immunosorbant assay (ELISA) on plates coated with the respective recombinant antigen used for immunisation. The challenge model proved highly reproducible between triplicate tanks in both trials. Despite enhanced specific antibody responses (>1/32) being detected in trypsin-vaccinated (Trypsin-VP2) salmon, no significant differences in chalimus numbers were noted between vaccinated and non-vaccinated fish following challenge. No significant differences in lice numbers were noted between vaccinated and non-vaccinated fish with any of the other 8 antigens tested. This trial showed that i.p. immunisation with these recombinant antigens at the dose used does not significantly reduce larval lice numbers despite enhanced specific antibody titres raised to some of the antigens.

References:
[1] Lees, F. *et al.* (2008) *Journal of Fish Diseases* **31**(12): 947-951;[2] Raynard S.R. *et al.* (2002) *Pest Management Science* **58**: 569-575; [3] Leclercq, E. *et al.* (2014) *Pest management science,* DOI: 10.1002/ps.3692.

Unravelling the acquired immune response to larval sea lice infections: a serological approach

S.J. Monaghan*[1], C.M. McNair[1], C. Metochis[1], K.D. Thompson[1], A. Adams[1], H.C. McDonald[1], S. Hamilton[2], D. Knox[2], R. Richards[1], P.D. Smith[3], W. Roy[4], T. Kanellos[5], D. Asper[6], J.E. Bron[1]

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[3]*Tethys Aquaculture, Ambo, Saffron Walden, Essex, UK*
[4]*Marine Environmental Research Laboratory, Machrihanish, Argyll, U.K.*
[5]*Pfizer Inc. (France), 75668 Paris Cedex 14, France*
[6]*Zoetis Aquaculture Biologics Research, Kalamazoo, MI, U.S.A*

The immune response of Atlantic salmon to *Lepeophtheirus salmonis*, has been extensively studied for different parasite life stages[1]. Parasite immuno-modulation inhibits the host pro-inflammatory response *e.g.* via IL-1β expression[2], which prevents larval success in other more resistant salmonid hosts *e.g*. coho or pink salmon[3,4,5,6]. Acute immune responses to larval sea lice have been well documented and earlier studies have shown that secondary challenges fail to elicit a decrease in parasite numbers relative to an initial challenge. However, the second challenge has often been conducted within a very short time-frame of the initial challenge[7]. The current study was carried out with re-infections of Atlantic salmon applied after a number of months (206 days post initial infection) to allow recovery of salmon from primary larval and adult infections, and thus potential louse-induced immuno-suppression, before exposure to the secondary wave of infection. Fish were sampled before second challenge (97 days after removal of initial infecting lice) and again post-challenge (14 days post re-infection) and immune responses compared to naïve fish treated the same way throughout the experiment. Blood plasma was sampled for serology to assess antibody response and complement activity. Immunoblots and ELISA were used to examine antibody recognition of louse antigens (fractionated extracts, recombinant proteins and secretions) and complement response was assessed with respect to alternative and classical pathways. The second challenge (*n*=20) resulted in very low (<10 lice; 25%) and very high (>150 lice; 50%) chalimus numbers despite identical challenge conditions, thus *post-hoc* comparisons were also carried out to elucidate any immunological relationships.

References:
[1] Fast M.D. (2014) *Developmental and Comparative Immunology* **43**: 300-312
[2] Fast M.D *et al.* (2007) *Parasite Immunology* **29**: 179-189
[3] Johnson S.C. & Albright L.J. (1992) *Diseases of Aquatic Organisms* **14**: 179-193
[4] Fast M.D. *et al.* (2002) *Diseases of Aquatic Organisms* **52**: 57-68
[5]Fast M.D. *et al.* (2007) *Fish and Shellfish Immunology* **22**, 403-407
[6] Jones S.R.M. *et al.* (2007) *Diseases of Aquatic Organisms* **75**: 229-238
[7] Frenzl B. *et al.* (2014) *Pest Management Science* **70**(6): 982-988

Secretory / excretory products of *Lepeophtheirus salmonis* regulate salmon leukocyte migration *in vitro*

J.L. Piesz*[1], I.R. Bricknell[1], Hernan Pizarro[2], S.E. Barker[1]

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[2]*FishVet Group, Portland, Maine, U.S.A.*

The salmon louse, *Lepeophtheirus salmonis,* evades host recognition by inhibiting inflammatory responses in Atlantic salmon; however, the mechanisms of this activity are not well characterized. Studies from other arthropod ectoparasites, such as ticks, have identified chemokine-binding proteins in the saliva and salivary gland extracts. These proteins inhibit neutrophil recruitment and activation *in vitro* and *in vivo* by interfering with chemokines, such as leukotriene B_4 (LTB4), preventing their interaction with receptors on the surface of neutrophils. To determine if *L. salmonis* is using similar mechanisms to evade the host immune response the effects of *L. salmonis* secretory / excretory products (SEPs) on LTB4 stimulated leukocyte migration *in vitro* using a 96-well chemotaxis chamber were investigated. Atlantic salmon peripheral blood leukocytes were exposed to LTB4 (1E-9M), and increasing doses of SEP's, or LTB4 and SEPs. Preliminary results showed a significant increase in leukocyte migration in response to LTB4 immune stimulation when compared to the control L-15 media. However, pre-incubation of LTB4 with SEPs for 1 hour reduced cell migration compared to LTB4 immune stimulation alone, and the response was dose dependent. These data suggest that *L. salmonis* may inhibit inflammatory responses in Atlantic salmon by secreting proteins with chemokine-binding or chemokine-degrading activity from their glands.

Tissue models for studying host-parasite interactions with salmon lice *Lepeophtheirus salmonis* (Copepoda, Caligidae)

H.C. McDonald*[1], A.P. Shinn[1], K.D. Thompson[1], K.F. Muir[1], S.J. Monaghan[1], C.M. McNair[1], R.H. Richards[1], D.P. Knox[2], S. Hamilton[2], D. Asper[3], T. Kanellos[4] J.E. Bron[1]

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[2]*Moredun Research Institute, Penicuik, UK;*
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[4]*Pfizer Inc. (France), 75668 Paris Cedex 14, France*

The use of integrated pest management strategies using veterinary medicines and a range of farm management tools has provided some control for sea lice in aquaculture, however a commercially viable vaccine to reduce infection would be highly advantageous. Current methodologies for study of vaccines and host immune responses involve the use of large numbers of fish; however, the development of *in vitro* Atlantic salmon tissue models would assist research on host-parasite interactions and contribute to reduction of animals in research. Fish scales could provide the structural support and tissue stability needed for maintenance of sea lice but there are limited studies into their use[(1-3)] or concerning sea lice maintenance *ex vivo*[(4)].

Investigations into the culture of scale-associated epithelial cells were undertaken, which examined various parameters in order to optimise culture conditions, as well as examining the effect of these on maintenance and survival of sea lice. From initial experiments, it was observed that caudal fish scales arranged in groups provided successful outgrowth of epithelial cells under standard tissue culture conditions and the use of collagen 1 and fibronectin were shown to enhance outgrowth. Preliminary survival of chalimus attached to scales was also achieved *ex vivo* for > 10 days and ongoing toxicity studies into environmental parameters will highlight any difficulties in current model development. Development of a viable culture technique that allows maintenance of sea lice larvae *in vitro* could provide a platform for investigation of localised host-parasite interactions. However, the conditions required to maintain sea louse viability must also be considered.

References:
[1]Akimoto, K. et al. (2000) Zoological Science, **17**(1): 61-63.
[2]Matsumoto, R. et al. (2007) Cell and Tissue Research, **327**: 249-265.
[3] Rakers, S. et al. (2011) European Journal of Cell Biology, **90**: 1041–1051.
[4]Butler, R. (2000) PhD Thesis, University of Stirling

Characterization and knock-down of a putative prostaglandin E synthase found in *Lepeophtheirus salmonis*

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[2]*Sea Lice Research Centre, Institute of Marine Research, Bergen, Norway*

The salmon louse (*Lepeophtheirus salmonis)* is able to modulate the immune defense of its host, the Atlantic salmon, causing a limited inflammatory response to lice infection. Prostaglandin E_2 produced by the louse is thought to be one of the main causes for this suppression. Prostaglandin E_2 is synthesized from its precursor by prostaglandin E synthase. In this study, a prostaglandin E synthase named LsPGES was isolated from the salmon louse. Expression of LsPGES was knocked-down in larvae by RNA interference and fish were infected with these modulated copepodids. Infection success and development of the lice was investigated. Difference in prostaglandin E concentration was measured in treated and control lice. In a time series of early stages the expression profile of the prostaglandin E synthase was investigated and localization was implemented in nauplius II, the stage with highest expression.

Increased susceptibility to infectious salmon anemia virus (ISAv) in *Lepeophtheirus salmonis* – infected Atlantic salmon.

S.E. Barker[1]*, J. Covello[2], D. Bouchard[1], W. Wolters[3], S. Purcell[2], M. Fast[2], I.R. Bricknell[1]

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The salmon louse and infectious salmon anemia virus (ISAv) are the two most significant pathogens of concern to the Atlantic salmon (*Salmo salar*) aquaculture industry. However, the interactions between sea lice and ISAv, as well as the impact of a prior sea lice infection on the susceptibility of the host to ISAv are still poorly understood. Therefore, this project aimed to determine a) whether sea lice take ISAv up from infected hosts, b) do sea lice remain infectious following host re-assortment, and c) are Atlantic salmon infected with sea lice more susceptible to ISAv and the impact(s) on their ability to respond to ISAv infection.

The data show that *L. salmonis* do appear to 'take up' viable ISAv when feeding on / infecting an ISAv positive Atlantic salmon host. *L. salmonis* were found to be positive for detectable viable ISAv at 16, 37 and 51 days post-exposure of the host fish to ISAv via a cohabitation challenge. They were found to be positive for viable ISAv up to four hours post-removal from the fish. Adult *L. salmonis* also appear capable of transmitting viable ISAv from an ISAv-infected Atlantic salmon to a naïve Atlantic salmon. When ISAv naïve salmon were infected with four adult lice per fish that had been removed from an ISAv-infected fish, 36.7% of naïve fish, were found to be positive for viable ISAv 30 days post-exposure (d.p.e.).

Finally, salmon pre-infected with late chalimus and pre-adult *L. salmonis* prior to ISAv exposure appear more susceptible to ISAv infection. Viable ISAv was detected earlier in cohabitee fish with a prior lice infection at 16 d.p.e. to ISAv compared to 37 d.p.e. to ISAv in the cohabitee fish without sea lice, and significantly lower survival was observed. Previous infection with sea lice appears to have some negative effects on the ability of Atlantic salmon to mount an appropriate early immune response to subsequent ISAv exposure, which will be discussed further. It is hypothesized that this may be due to the fact that the immune response is already geared towards responding to the sea lice infection, potentially leading to delayed viral recognition and activation of an appropriate early immune response to ISAv.

Transcriptomic evidence for host-specific feeding responses of *Lepeophtheirus salmonis*

L.M. Braden*[1], B.J.G. Sutherland[1], B.F. Koop[1], S.R.M. Jones[2]

[1]*University of Victoria, Victoria, B.C., Canada*
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Infections with the ectoparasitic copepod, *Lepeophtheirus salmonis*, result in a variable inflammatory host reaction at the louse attachment site that is enhanced in resistant species (coho salmon, *Oncorhynchus kisutsch*), but which is reduced or absent in susceptible species (Atlantic salmon, *Salmo salar*). This research explores the molecular basis of this variable host reaction, with emphasis on the responses at the attachment site by both the parasite (feeding, attachment) and the host (defense). *L. salmonis* secretes various compounds such as prostaglandin-E_2 and trypsin proteases, which act to suppress host inflammatory responses and aid in feeding. To test the hypothesis that *L. salmonis* exhibits species-specific feeding responses, we applied a 38K oligonucleotide microarray to profile the parasite transcriptome during attachment / feeding after 24 and 48hrs on Atlantic, coho and another susceptible species, sockeye salmon (*O. nerka*). The lice were observed to feed on all species; however, functional enrichment analysis of gene expression data indicated responses were influenced by the host. Expression profiles of genes involved in feeding (*e.g.* proteases) and energy production (*e.g*. cytochrome oxidases) were observed to increase over time during attachment on Atlantic salmon, while on coho or sockeye salmon the response decreased and more closely resembled profiles viewed in starved *L. salmonis* after 48hrs. Together these data suggest that *L. salmonis* exhibits species-specific feeding responses, and further, that these responses may contribute to host-susceptibility.

Profiling the effects of plant derived anti-lice bioactives on salmon louse and Atlantic salmon

S. Skugor*[1], H. Holm[1], A.K. Osmo[2], T. Utne[2], A. Krasnov[3], Ø. Evensen[1], S. Wadsworth[2]

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[2]*EWOS Innovation, Dirdal, Norway*
[3]*Nofima, Ås, Norway*

Inclusion of anti-lice plant bioactives in feed represents a promising approach in the integrated control of *L. salmonis*.

Our microarray screening revealed transcriptome changes upon sensing Atlantic salmon mucus. Next, we showed that these early responses, likely involved in the attachment of infective copepodids, can be modulated by exposure to water-soluble anti-lice compounds. Incubation with these plant derived anti-attachment bioactives resulted in less effective attachment to pelvic fin tissues *ex vivo* and affected the expression of several mucus-responsive genes and genes that are normally regulated during the transition period from free swimming to attached parasitic stages.

In our pipeline, in addition to direct effects on the parasite, candidate anti-lice bioactives are evaluated for their *in vivo* efficacy, that is, when administered in feed. Several challenge trials with varying doses of bioactive compounds ratified reduction in lice counts and thus efficacy of oral delivery while screening salmon skin by microarrays revealed induction of responses, notably immune, that could at least partly explain the diet-mediated protection.

Novel feed ingredients must also meet the requirement of minimal toxicity to the host. To address this issue and to investigate other possible protective modes of action of plant anti-lice compounds we looked at responses in tissues involved in important immune, metabolic and detoxifying functions. Comprehensive gene expression profiling of liver, muscle and distal kidney was complemented with blood analysis and immunohistochemistry. Health-promoting effects of studied bioactives, including their pro-apoptotic, antioxidant and detoxifying capacities, will be discussed in relation to their potential role during lice infection.

Development of a vaccine against sea lice

Yamila Carpio González[1], Claudia García Castillo[1], Juana Maria Lugo González[1], Jannel Acosta Alba[1], Liliana Basabe Tuero[1], Antonio Morales[1], Reynold Morales[1], Osmany Rodrigo[1], Fidel Herrera[1], Janet Velazquez[1], Alexis Machin[1], Yeny Leal[1], Mario Pablo Estrada[1]*

[1]*Animal Biotechnology Division, Center for Genetic Engineering and Biotechnology, Havana, Cuba*

Sea lice (Copepoda, Caligidae) are the most widely distributed marine pathogens in the salmon industry. Vaccination could be an environmentally friendly alternative for sea lice control, however, research on the development of such vaccines is still at an early stage of development. Recent results have suggested that subolesin/akirin/my32 are good candidate antigens for the control of arthropod infestations, including sea lice, but background knowledge about these genes is limited. We characterize the my32 gene/protein from two important sea lice species, *Caligus rogercresseyi* and *Lepeophtheirus salmonis*, based on cDNA sequence isolation, phylogenetic relationships, three dimensional structure prediction and expression analysis. The results show that these genes/proteins have the main characteristics of akirins from invertebrates. Immunization with purified recombinant my32 from *L. salmonis* elicited a specific antibody response in mice and fish. We also studied different strategies to improve vaccination response such as fusing my32 to another physiologically relevant antigen and the use of endogenous molecules as molecular adjuvants. These results provide an improvement to our current knowledge about potential vaccine candidates against sea lice in fish.

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Notes

Notes

Friday – Plenary Talk

G.A. Boxshall

Department of Life Sciences, The Natural History Museum, Cromwell Road, London SW7 5BD, United Kingdom

SEA LICE AS COPEPODS

Copepods are one of the three mega-abundant metazoan life forms on Earth and nearly half of all described marine copepods live as parasites in association with metazoan hosts. Comparative studies on free-living and other parasitic copepods have been valuable in informing research on sea lice. For example, it was insights from comparative biology that stimulated the recent revision of the *Lepeophtheirus salmonis* life cycle and brought it into alignment with the life cycle of all other copepods. Such comparative approaches allow us to identify aspects of sea lice biology which may be worthy of more detailed investigation in the search for effective control methods. Such aspects include: 1, the chemically-mediated component of host location behaviour; 2, the expression of host specificity; 3, the pheromones involved in mate location behaviour; 4, the development of the frontal filament in chalimus larvae, 5, the development of lunules in *Caligus*-like genera, and 6, the form of the nauplius stage. Research into several of these areas of sea lice biology has been patchy, and some have been largely ignored. I would, for example, have expected a greater focus on host specificity, the extent to which a parasitic species can exploit different host species. This is a fundamental property of parasites and is a key measure of the ability of a parasite to colonise novel hosts. The areas of research identified here are promising because they can be focussed on sea lice, or on a wider group of related fish parasites. This taxon focus is critical.

Notes

Posters

Poster #1

Time series models of Sea lice (*Caligus rogercresseyi*) abundance on Atlantic salmon and rainbow trout in Chile

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Caligus rogercresseyi is one of the most important challenges of the salmon industry in Chile. Since June 2013, Aquabench S.A. has been working with 15 salmon producers, in a Caligus Coordinated Control Plan, with the purpose to maintain the infestation levels under control. In this scenario and with the objective of understanding the behavior of this parasite in recent years, a time series analysis of abundance of *Caligus rogercresseyi* gravid females in Atlantic Salmon and Rainbow Trout was carried out using data from January 2010 to March 2014, in the main areas of salmon farming in southern Chile. As a result of this analysis (Fig.1) a seasonal index of sea lice abundance was calculated and showed the greatest differences from the average were recorded in March (0.86), January (0.79), September (-0.37) and August (-0.33).The trend analysis showed a steady increase in tparasite abundance starting in 2012, with a peak in autumn 2013 and a decreasing curve by 2014. Finally, an autocorrelation analysis determined there would be two autoregression periods (significant correlation different from zero). In conclusion, we can establish that seasonality in the abundance of gravid female of *Caligus rogercresseyi* exists, with higher levels in the autumn months (March and April) and lower abundances in September and August. Additionally the infestation levels of a specific time was associated with the two previous periods.

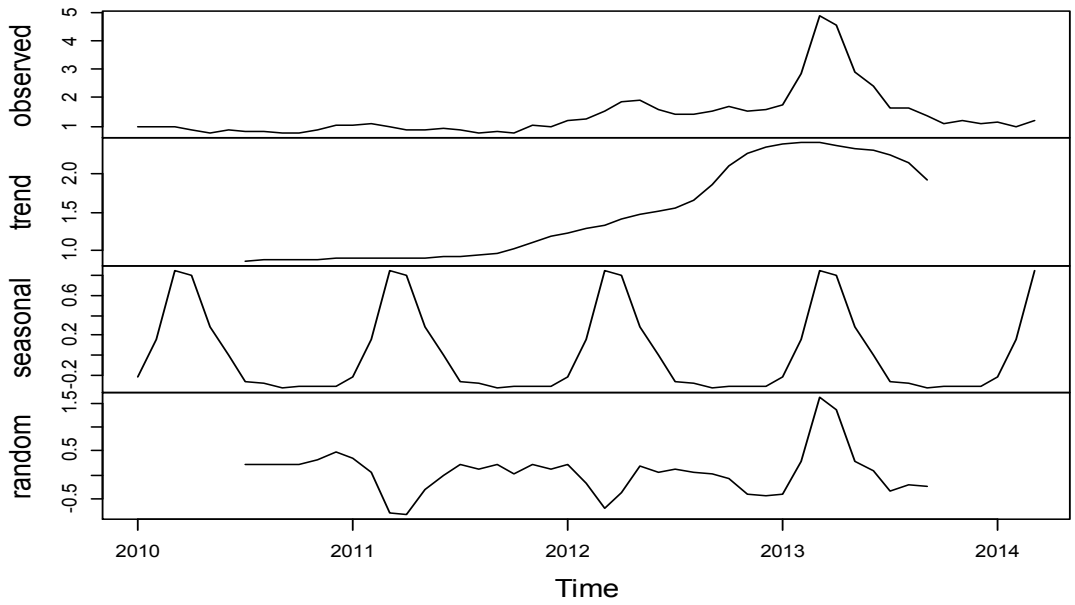

Fig1.
Decomposition of additive time series of *Caligus rogercresseyi* abundances in Chile from January 2010 to March 2014.

Poster #2

Salmon lice challenge model

M.S.W. Breiland[1]*, H. Mikalsen[1], A. Krasnov[1] and L.-H. Johansen[1]

[1]*Nofima (Norwegian Institute of food, fisheries and aquaculture), Tromsø, Norway*

The Salmon louse (*Lepeophtheirus salmonis*) is the most significant parasite in salmon farming in Europe, and it has also become a huge problem for Norwegian aquaculture. We started working with this parasite in 2010 in cooperation with the Aquaculture research station in Tromsø. Since then we have run several internal pilot studies and established a salmon lice facility and a reproducible salmon lice challenge model. Important factors to know and control are challenge dose and challenge time, fish size, water temperature and when to count lice and end an experiment. To get the desired number of lice per fish, it is deciding to know the number of copepodites per fish to use for challenge. It is also important to know the different developmental stages of the lice to avoid the stages when the lice start to move and leave the fish. A study in our lice facility showed a reduction of lice in fish fed with sex hormones. Transcriptomic analyses revealed differences between groups and knowledge was gained on factors contributing to lice resistance. Results from this study will be presented. In the lice laboratory we also have the opportunity to study lice-infected salmon together with lumpfish in controlled set-ups. Lumpfish (*Cyclopterus lumpus*) are used as cleaner fish for reducing the amounts of salmon lice in Atlantic salmon farms. However, much knowledge is still lacking on the co-existence of salmon and lumpfish and the abilities of lumpfish to eat salmon lice.

Poster #3

Salmon louse development in summer temperatures

E.P. Garcia[1], L. Asplin[2], S. Dalvin[1]*

[1]*Sea Lice Research Centre, Institute of Marine Research, Bergen, Norway*
[2]*Institute of Marine Research, Bergen, Norway*

The salmon louse is the main parasite of concern in salmonid aquaculture production in Norway. Production of salmon lice from fish in aquaculture is considered a significant threat to wild fish (Atlantic salmon and sea trout). Management and preservation of wild salmon stocks in Norway entail quantification and modeling of salmon lice populations to estimate infection pressure. The developmental rate of salmon lice is greatly influenced by water temperatures, likewise is their reproductive output. Salmon louse larvae are released from adult female lice as nauplius larvae and are transported by the current whilst they develop into copepodites, the infectious stage. Currently, we estimate that larvae are infectious between 50 and 150 degree days (Asplin *et al*. 2011 and references within), but this remains an estimate when looking at temperatures outside the range where the original experiments were performed. To improve the accuracy of modeling, we need precise measurements of the developmental rate as a function of temperature. The goal of this pilot-study was to obtain more accurate numbers for how long it takes for the salmon louse to develop into adulthood and for how long copepodites survive. The experiment was performed at 15 degrees, which is a temperature likely to be experienced by salmon louse over a longer period in the summer in fjords on the west coast of Norway, an area with extensive aquaculture activities.

References

L. Asplin, K. Boxaspen, A.D. Sandvik. 2011. Modeling the distribution and abundance of planktonic larval stages of *Lepeophtheirus salmonis* in Norway. In: Jones SRM, Beamish RJ, editors. Salmon Lice: An Integrated Approach to Understanding Parasite Abundance and Distribution. Hoboken, NJ:Wiley-Blackwell, p 3150.

Poster #4

Can the explanation of pyrethroid resistance in salmon lice (*Lepeophtheirus salmonis*) be found in the *para* type voltage gated sodium channel?

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[2]*University of Bergen, Sea Lice Research Centre, Bergen, Norway*

Pyrethroid resistance in the salmon louse *Lepeophtheirus salmonis* is a major concern in the Norwegian fish farming industry as most fish farmers are dependent on chemical treatments in order to keep the lice amount below permitted numbers. The mechanism for pyrethroid resistance in salmon lice is not yet known, although it has been revealed in several other species. One of the most commonly seen resistance mechanisms against pyrethroids are target-site mutations in the *para* type voltage gated sodium channel gene. The aim of the current study was to isolate this gene in *L. salmonis* and to search for mutations in the gene among pyrethroid resistant parasites from different parts of Norway.

The gene was fully sequenced using RACE PCR. Homologous sequences to hot-spots for mutations known from other species were identified. 47 salmon lice from four different areas of Norway, all survivors of deltamethrin laboratory-treatments, were investigated for mutations in these hot-spots using PCR and direct sequencing.

No mutations were identified when compared to the sequences collected from a fully sensitive strain of salmon lice. However; three silent base changes were found in the sequences included in the study. These changes displayed a great heterogeneity among the studied sea lice. The two findings put together indicate that target-site mutation is not the mechanism behind pyrethroid resistance in salmon lice in Norway, and that future research for resistance mechanisms should be targeted elsewhere.

Poster #5

Hatching of salmon louse egg strings after 30 min exposure to various pharmaceutical compounds

S.M. Aaen[1]* and T.E. Horsberg[1]

[1]*Norwegian University of Life Sciences, Sea Lice Research Centre, Oslo, Norway*

Existing anti-parasitic compounds used in salmon farming are directed to be effective against defined instars. Hydrogen peroxide is the only compound reported to be effective against hatching of salmon lice egg strings. Preventing the parasite's reproduction would offer a beneficial tool to reduce the sea lice abundance. Therefore, the effect of several compounds on egg string hatching was monitored in a series of experiments.

For each compound, 12 egg strings were cut in two. One moiety was exposed to the medicinal compound and the other moiety was treated as a control. The concentration, 50 mg/mL, was chosen to be similar to the one used in parallel screening experiments with preadult salmon lice. Each egg string was exposed for 30 minutes, rinsed and then incubated in running sea water for up to 540 hours, with the number of larvae registered every 48-72 hours. Area Under Curve (AUC) was calculated for each compound, and compared with control AUC.

Most of the compounds did not affect the hatching to a significant degree. Only emamectin benzoate and cypermethrin proved to possess oocidal properties. The following compound classes were examined:

Carbamates	Organophosphates	Fenylpyrazoles	Pyrethroids
Neonicotinoids	Avermectins	Ecdysone agonists	Feeding blockers
Phosphate uncouplers	Juvenile hormone analogues	Chitin synthesis inhibitors	Molting disruptors
Nicotinic acetylcholine receptor blockers	Pyrazin-isoquinolone derivates	Oxydative phosphorylation modulator	Acetyl coenzyme A carboxylase inhibitor
Sodium channel blockers	Ryanodine receptor modulators	Octopamine receptor agonists	Unknown mechanism

Poster #6

Vertical salmon lice behavior as a response to environmental conditions and its influence on the regional dispersion in a fjord system

Ingrid A. Johnsen[1,*], Øyvind Fiksen[2], Anne D. Sandvik[1] and Lars Asplin[1]

[1]*Institute of Marine Research, Bergen, Norway*
[2]*Department of Biology, University of Bergen, Bergen, Norway*

The salmon louse (*Lepeophtheirus salmonis)* is a major parasite of salmon, and is able to travel between farms through its pelagic phases. We investigated the spatial dispersion of planktonic stages in the Hardangerfjord, Norway, using an individual-based model and a fjord circulation model. The models allow us to investigate how assumptions about swimming responses to environmental cues affect vertical distribution, development and horizontal transport. The rules governing vertical distributions include remaining fixed at constant depths but also prescribed responses to environmental cues such as ambient light level, salinity and temperature. The horizontal dispersion was affected by the vertical distribution scheme (rules) of the particles. When particles were held fixed in the surface layer the horizontal dispersion and the area potentially affected by a source of lice decreased relative to distribution predicted when lice had vertical migration behaviours. The simulations also showed that swimming triggered by both light and temperature may result in a diel migration pattern. However the diel migration during the copepodid stage did not influence the horizontal distribution in May for this particular area, but the assumptions and implementation of small-scale vertical habitat selection often play a major role in both the direction and extension of the dispersal. Better information of the actual response lice have to a set of vertical environmental factors is needed to improve predictions of lice dispersal in fjords.

Poster #7

Developmental stages and fecundity of *Lepeophtheirus simplex* parasitic on bullseye puffer fish (*Sphoeroides annulatus*)

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[2]*Instituto de Ciencias del Mar y Limnología, UNAM, Mazatlán, Sinaloa, México*

Lepeophtheirus simplex is a parasite of *Sphoeroides annulatus*, an economically important fish species, with potential for aquaculture, in northwestern Mexico. The goal of this study was to describe the developmental stages of this parasite under experimental conditions as well as its seasonal fecundity on wild fish. There are two naupliar, one copepodid, two chalimus, and two pre-adult stages preceding the adult of *L. simplex*. The results support previous findings, which point out that the life cycle of the caligid copepods only includes six post-naupliar stages. The generation time from egg extrusion to adult for *L. simplex* was approximately 10 days at 22 °C. The body length of the ovigerous females ranged between 2.2 and 4.1 mm, and its fecundity between 12 and 36 eggs per string. Fecundity was negatively correlated with the egg size and positively correlated with the egg string length. Our data did not reveal significant differences in fecundity among sampling months, but ovigerous females were significantly larger in March (when water temperature was 22°C) than in June and July (when water temperature was 30°C). To some extent, our fecundity results contrast with those found in sea lice species from higher latitudes. Nonetheless, biological information on different sea lice species from different environmental conditions is necessary in order to understand their infection strategies, which could be useful in view of the increasing interest in marine fish farming in Mexico.

Poster #8

Generating knowledge to optimize bath treatments against *Caligus rogercresseyi* and development of a novel tool for knowledge transfer into the Chilean salmon industry

M.A. Hausdorf[1]*, J.J. Aurtenechea[1] and J.E. Unibazo[1]

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Antiparasitic bath treatments are a common technique used to control *Caligus* in Chile. From 2013, the authorities imposed regulations that required all treatments by immersion to be performed in closed systems. At that point, the required methodology to perform efficient bath treatments was not well defined, and the available technology was not specifically designed to handle the materials and equipment involved. Our project aimed to generate and transfer the necessary knowledge for reducing the identified operational and technological gaps. For this, we organized a technical committee, comprising salmon producers, equipment and materials suppliers and experts in the fields of fish health, engineering and aquaculture operations. Based on field observation of bath treatments, the technical committee analyzed and discussed current practices and further identified operational and technology gaps. Results showed that important items to be checked before performing bath treatments are: (1) cage characteristics, (2) environmental conditions, (3) production and fish health conditions, (4) availability of required materials and staff. Furthermore, we identified six critical operational steps: (1) loosening and lifting the fish net, (2) oxygen system and monitoring, (3) tarpaulin installation, (4) chemical preparation, distribution and exposure time, (5) tarpaulin removal and (6) fish observation and water exchange. Optimized operational procedures and recommendations were presented to the salmon industry in an interactive good practices manual. We concluded that operational and technological gaps can be identified and minimized by generating the required knowledge, and that the use of innovative user friendly tools facilitates the transfer of such knowledge.

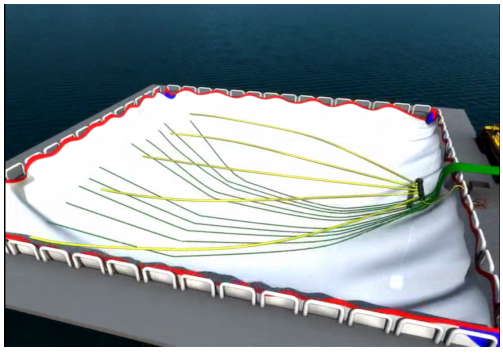

Figure 1. 3D image of a fish cage with all the materials installed to perform a bath treatment.

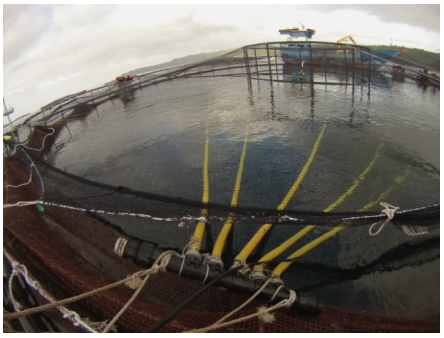

Figure 2. A fish cage with the chemical distribution system installed to perform a bath treatment.

Poster #9

A protocol for RNAi induction in early salmon louse stages

C.M.A. Caipang[1] and R. Skern-Mauritzen[2]*

[1]*Temasek Polytechnic, Singapore, Singapore*
[2]*Institute of Marine Research, Bergen, Norway*

The salmon louse is a significant pathogen of wild and farmed salmonids in the northern hemisphere, and the economic burden they inflict has resulted in increasing molecular research and methodological advances on the species during the last decade. Among the methodological advancements has been the use of RNAi, among other things for the search of potential vaccine antigens. Inducing RNAi in early stages has hitherto been problematic, and a reliable protocol has not been available until recently (Dalvin *et al.* 2014). However the published protocol requires accurate staging of the nauplii used and is effective only on nauplii and onwards. We present here an improved protocol based on lipofection that allows induction of RNAi in nauplii irrespective of developmental advancement and furthermore this technique is even more effective when applied to eggstrings. The presented protocol is thus superior in that it is easier to apply to larval stages and also allows downregulation of genes in the embryonic stage.

Poster #10

Understanding underlying processes and requirements for hydrodynamic models used for sea lice dispersal: a Scottish sea loch application

B. Rabe[1], N.K.G. Salama[1]*, J. Wright[1] and C.M. Collins[1]

[1]*Marine Laboratory Aberdeen, Aberdeen, UK*

Models are useful tools for understanding environmental transmission of disease-causing agents from aquaculture sites (Salama and Rabe, 2013). To successfully run coupled bio-physical models to study sea lice dispersal, an understanding of the underlying dynamics of the environment is needed. This information feeds into hydrodynamic models for which certain questions need to be answered: What data are required to force and validate the hydrodynamic model before coupling it with the biological model? What physical principles and processes in the system, like buoyancy- or wind-driven flow, is it essential to represent?

An example of on-going sea lice research in Scotland focuses on Loch Linnhe, a 60km long sea loch on the west coast. A regular-grid hydrodynamic model was forced with field-measured winds, climatological freshwater input, meteorological conditions, temperature, salinity, and tides. The model predicted observed tides and currents during validation periods well. The results highlighted the complex oceanography within the loch. The 5km wide loch is dynamically wide, *i.e*. the Earth's rotation has a noticeable effect, with a rotational length scale of ~2km. Sea lice dispersal is therefore affected by winds, tides, freshwater outflow and rotational effects. All of these factors will need to be evaluated to provide a realistic hydrodynamic model to be coupled to the biological sea lice dispersal model to avoid negative consequences such as impacts on predictions and influences on outcomes and advice. The outcomes of this research are likely to have direct applications on disease management and sea lice treatment strategies, especially in wide fjordic systems.

References:

Salama N.K.G. and Rabe B. 2013, Developing models for investigating the environmental transmission of disease-causing agents within open-cage salmon aquaculture, *Aquacult. Environ. Interact.* 4:91-115

Poster #11

Protein identification in *Lepeophtheirus salmonis* extracts with the potential for selection of vaccine candidates

S. Hamilton[1]*, S. Monaghan[2], C. McNair[2], W. Roy[2], A. Adams[2], K. Thompson[2], R. Richards[2], T. Kanellos[3], D. Asper[3], J.E. Bron[2] and D.P. Knox[1]

[1]*Moredun Research Institute, Midlothian, Scotland*
[2]*Institute of Aquaculture, Stirling, Scotland*
[3]*Zoetis, Kalamazoo, United States*

Background: The salmon industry in Scotland accounts for £1 bn in contributions to the United Kingdom economy. The cost associated with controlling the sea louse parasite *Lepeophtheirus salmonis* (*L. salmonis*) is estimated at £33m per annum. There is therefore a need for a novel, effective and environmentally friendly solution to reduce *L. salmonis* numbers in farmed salmon.

Aim: Our aim is to produce a vaccine which will reduce sea louse numbers in farmed salmon and consequently contribute to sustainable protein production in the UK and worldwide.

Materials and Methods: Four main approaches were used: 1. Louse tissue sections were probed with fluorescently labelled lectins to identify potential sites of interest. 2. Enzymatic profiles of louse extracts were examined using Zymogram gels. 3. Western blotting was used to determine immuno-reactive proteins present in louse extracts. 4. Affinity chromatography was used to enrich for proteases and lectin bound proteins. Secretions / excretions were also collected from lice and examined using mass spectrometry.

Results: Three lectins were selected for further use based on their binding to gland-like structures in the louse. Substrate gels revealed the presence of proteases in louse extracts. No distinct immunogenic protein bands were observed on western blots. Louse fractions obtained via affinity columns were used in a vaccine trial.

Discussion: Results from the vaccine trial are presented. Further trials will be optimised pending current trial results. From the mass spectrometry data collected, recombinant proteins will be produced and trialled in salmon.

Poster #12

Catch me if you can

M. R. Pietrak[1]*, S. D. Molly[1] and I. R. Bricknell[1]

[1]*University of Maine, Orono, Maine USA*

Sea lice (*Lepeoptheirus salmonis*) have been a parasite of concern in salmon farming for many years leading to years of work by growers and researchers to better understand this pest. Due to the obligatory parasitic nature of *L. salmonis* the development of challenge methods to infect host fish with copepodids allowing the parasite to develop and complete its lifecycle in captivity has been incredibly important. Current challenge models have allowed researchers to better understand host-parasite interactions and test new treatment compounds, along with making many other studies possible. All of the current challenge models rely on exposing copepodids to potential hosts for some period of time in a confined volume. Refinements to aspects such as the number of copepodids, volume of water, or the length of exposure in order to better mimic various aspects of a natural infection has occurred. This work describes refinements to the basic challenge model allowing for experiments to evaluate the success of traps or lures in reducing the infectious pressure. Trials conducted demonstrated that mussels placed upstream of salmon such as on an IMTA site, have the potential to reduce the infectious pressure on fish downstream. A series of raceways were established with constant flow rates allowing copepodids to be placed upstream of simulated mussel rafts, and then pass on to infect hosts in a single pass. The system was established with a single pass flow-through design so that copepodids that did not successfully find a host before being carried out of the system were not given a second chance to infect a host. The studies showed that mussels were capable of reducing the infectious pressure part of the time.

Poster #13

Evidence from the field of mussels ingesting larval sea lice on an integrated multi-trophic salmon farm

M. R. Pietrak[1]*, S. D. Molly[1] and I. R. Bricknell[1]

[1]*University of Maine, Orono, Maine USA*

Sea lice (*Lepeoptheirus salmonis*) have been a parasite of concern in salmon farming for many years. Their populations are commonly managed through the use of various chemotherapeutants in the various salmon farming regions of the world. A few non-chemotherapeutant based techniques such as the use of cleaner fish, fallowing and reduced stocking methods are used as well to help manage this important parasite. Recently, several groups have provided lab-based evidence that blue mussels (*Mytilus edulis*) and other shellfish species are capable of ingesting planktonic stages of sea lice. This is the first report of field-based evidence that mussels co-cultured on an Integrated Multi-Trophic Aquaculture (IMTA) site are ingesting sea lice. Stomach contents from 50 mussels haphazardly selected from culture lines on an IMTA site were sampled for the presence of the mitochondrial cytochrome oxygenase I (mtCOI) gene from *L. salmonis*. Of the 50 mussels sampled the stomach contents from one was positive for mtCOI. This finding represents the first field-based evidence as far as we are aware of blue mussels consuming sea lice. This evidence suggests that lab-based indications of the potential for IMTA to reduce sea lice populations on co-culture salmon merit further field investigations.

Poster #14

Lepeophtheirus salmonis 200k SNP chip

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Ectoparasites, such as *Lepeophtheirus salmonis*, are a prevalent point of discussion in salmonid research. They exhibit marked differences in populations. With the goal of shedding some light about the characteristics of these differences, we designed a ~200k SNP chip for *L. salmonis*. We sequenced the genome of 38 individuals encompassing both Atlantic and Pacific populations. Atlantic *L. salmonis* individuals comprised SLICE resistant/non-resistant males and females, while Pacific *L. salmonis* individuals comprised adult males and adult females from various geographic locations. In a preliminary analysis, SNPs were identified from separate Atlantic and Pacific populations. We found that Pacific populations showed a lower ratio of heterozygous : homozygous SNPs. Pacific populations had twice (avg. 6 M) as many heterozygous SNPs as the Atlantic populations (avg. 2.3 M). A microbial metagenome analysis for each individual louse is underway.

201,279 SNPs were tiled on an Affymetrix Axiom SNP chip. SNPs were chosen to be able to answer questions regarding both Pacific and Atlantic populations. Group 1 SNPs (~2.5%) combined SNPs from a previous design, and includes a new marker for sex. Group 2 SNPs (~2.5%) represent those that are identified as either unique in either SLICE resistant or SLICE sensitive groups. Group 3 SNPs (~31%) are found in either Atlantic, Pacific, or both populations. These SNPs are present in exonic regions, spaced approximately 8 kbps apart. Group 4 SNPs (~48%) represent non-transcribed regions in Atlantic populations, and are spaced approximately 4 kbps apart. Lastly, Group 5 SNPs (~16%) represent low frequency (potentially recent) SNPs, appearing in less than 30% of Atlantic individuals and spaced 10 kbps apart. This finalized set of SNPs has been printed, and is presently undergoing validation. We expect to have a preliminary test set of results for this SNP chip. If successful, it will provide a powerful new tool for understanding salmon louse biology.

Poster #15

The use of lumpfish (*Cyclopterus lumpus* L.) To control sea lice (*Lepeophtheirus salmonis* (Krøyer)) infestations in intensively farmed Atlantic salmon (*Salmo salar* L.)

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*Lepeophtheirus salmonis (*Krøyer) has been reported to cause alterations in physiological homeostasis and mortality in salmonids (Sivertsgard *et al.* 2007).
Today, the density of lice is mainly regulated with the aid of chemotherapeutants, but increasing resistance to delousing agents is an emerging problem (Jimenez *et al.* 2012). The biological control of sea lice through the use of cleaner fish has recently become a feasible option due to the urgent need for an effective method of parasite control in Atlantic salmon aquaculture. Currently, wrasse species in use for biological delousing are temperature sensitive, making them unfit for use at temperatures less than 6°C (Sayer and Reader, 1996). As a cold-water alternative, the common lumpfish (*Cyclopterus lumpus*) has been suggested.

To assess the efficacy of lumpfish grazing on attached sea lice from Atlantic salmon a duplicate study was undertaken.

There were clear signs of lumpfish grazing on sea lice, with significantly lower average numbers of pre-adult, mature males and females stages of *Lepeophtheirus salmonis* per salmon. Lumpfish reduced the mature female stage of *L. salmonis* to levels equal to or lower than the counts recorded prior to the start of the study. There were no significant differences between the treatments (10% and 15% densities) in grazing efficacy.

Overall, the present results indicate that lumpfish is a suitable cold-water option for biological delousing of Atlantic salmon.

References

R. Sivertsgard, E.B. Thorstad, F. Okland, B. Finstad, P.A.Bjorn, N. Jepsen, T. Nordal, R.S.McKinley 2007. Effects of salmon lice infection and salmon lice protection on fjord migrating Atlantic salmon and brown trout post-smolts. *Hydrobio* 582:35–42.

D.F.Jimenez, P.A.Heuch, C.W. Revie, G. Gettinby 2012. Confidence in assessing the effectiveness of bath treatments for the control of sea lice on Norwegian salmon farms. *Aquaculture* 344:58–65.

M.D.J. Sayer, J.P. Reader 1996. Exposure of goldsinny, rock cook and corkwing wrasse to low temperature and low salinity: survival, blood physiology and seasonal variation. *J Fish Biol* 49:41–63.

Poster #16

A transcriptomic comparison of copepodid and pre-adult sea lice (*Lepeophtheirus* salmonis)

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Transcriptomic evaluations of *L. salmonis* have primarily focused on drug responses of adult stages of *L. salmonis,* however, little has been done to characterize differences between life stages. The present study applied a 38K oligonucleotide microarray to analyze the transcriptional profiles of copepodid and pre-adult *L. salmonis* from the same cohort. As recent studies have elucidated the complexity of the *L. salmonis* transcriptome and variability across planktonic cultures, it is essential to understand the developmental processes of this parasite through all of its life stages with the same genetic background. Approximately 6000 copepodids were cultured in the lab from parents originally from Grand Manan, New Brunswick, in the Bay of Fundy, Canada. Half of these parasites were collected and flash frozen while the remaining copepodids were exposed to Atlantic salmon (*Salmo salar*), and allowed to develop to the pre-adult life stage. Approximately 6700 genes were differentially expressed ($p<0.01$; Benjamini-Hochberg MTC, fold change>1.5), 2539 of which are not annotated. Genes upregulated in copepodids compared to pre-adults were enriched for biological regulation (279 genes), generation of precursor metabolites and energy (79 genes), ion binding (203), and synaptic development (23 genes). Conversely, genes upregulated in pre-adults were enriched for carbohydrate metabolism (50 genes), extracellular space (24 genes), catalytic activity (395 genes), vitamin B6 binding (20 genes) and pyridoxal phosphate binding (15 genes). This same cohort was also exposed to anti-sea lice treatments (emamectin benzoate, cypermethrin, etc.) and drug responses compared across life stages. The present study offers a global transcriptomic snap-shot of *L. salmonis* development and drug response between free-swimming and parasitic life stages, from the same cohort.

Poster #17

Using management data to describe the epidemiology of sea lice in Scotland

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Collection of data on sea lice for scientific purposes is expensive and time consuming. It is therefore cost-effective to analyse data that are being collected for management purposes. Two such data sets have been used to provide descriptive epidemiologies of sea lice in Scotland. These are the treatment data collected by the Scottish Environment Protection Agency (SEPA) to monitor discharges, and lice count data collected by the Scottish Salmon Producers' Organisation (SSPO) and published as area average counts on a monthly basis. The SEPA data set has been combined with a catalogue of sea lochs (small fjords) to model hydrographic factors associated with treatment rate. The key factors are region, total biomass of farms in the sea loch and flushing time of water in the sea loch. However, the relationship with flushing time depends on the region, treatment increasing with flushing time on the mainland, but decreasing on the Western and Northern Isles. Sea lochs, rather than individual farms, are appropriate units for assessing treatment requirements, which depend on larger oceanographic processes. The SSPO data have been used to develop a descriptive analysis of sea lice counts in Scottish salmon farms. Lice abundance shows strong seasonal and spatial patterns, with most areas exhibiting low counts and a few areas consistently returning counts in the upper quintile. These analyses are the first for Scottish sea lice epidemiology that cover 95% of production from all salmon farming areas.

References:

A.G. Murray & M. Hall 2014 Treatment rates for sea lice of Scottish inshore marine salmon farms depend on local (sea loch) farmed salmon biomass and oceanography. Aquacult. Env. Interact. 5, 117-125

Poster #18

Rapid assessment of potential dietary anti-lice compounds *in vitro*

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Sea lice remain a major problem in salmonid aquaculture. Increasing reports of resistance to, and environmental concerns regarding, current chemical control methods have resulted in the search of alternative and more environmentally friendly approaches. One such approach to sea lice control is via dietary intervention. The necessary R&D dietary studies usually involve *in vivo* fish trials, but when investigating anti-sea lice diets, *in vitro* methods may be used to pre-screen a number of potential microingredients. Previous studies have used electrophysiological methods and Y-tubes to test a sea louse's response and preference or deterrence to various substances. Presented here is an alternative *in vitro* method that allows a number of copepodids to be monitored in real-time both qualitatively and quantitatively.

Poster #19

Assessment of the repellant activity of anti-parasitic compounds to larval stages of *Lepeophtheirus salmonis*

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The salmon louse, *Lepeophtheirus salmonis*, represents one of the greatest disease challenges facing the global Atlantic salmon industry. Resistance developed in lice to a range of current medicines, *e.g.* emamectin benzoate (SLICE®)[1], emphasises the need for alternative, environmentally friendly control methods.

The aim of this study was to find a compound that is effective at repelling lice at low concentrations, this minimising the level of release into the environment. Twenty-four hour toxicity tests were performed in 24-well plates containing copepodids (n=5 well^{-1}) ≤3 days post moult from the nauplius II stage. Seven water soluble compounds were tested, three of which, were highly soluble in seawater (two plant extracts (herb 4 & 6) and one synthetic cosmetic ingredient (synth 1) and suitable for use at concentrations of < 31.25 µg mL^{-1} and 62.5 µg mL^{-1}, respectively for herb 4 and 6, and 1.9 µL mL^{-1} for synth 1.

Known salmon kairomones[2,3] were used as positive controls in a flow-through, Y-shaped choice chamber to attract larval lice. The three candidate repellent compounds were each mixed with kairomones in one arm in an attempt to inhibit chemotaxis of the lice to the kairomones. To assess the repellent potential of each compound, the behaviour of the lice in the choice chamber was recorded using a video camera. The movements of the lice were subsequently digitised and quantified using *Paratrack* parasite tracking software (Brooker, University of Stirling[4]) enabling any significant preference of lice for one of the arms of the choice chamber to be determined.

References:
[1]Lees F. *et al.* (2008) *Journal of Fish Diseases* **31**(12): 947-951
[2]Ingvarsdóttir A. *et al.* (2002) *Pest Management Science* **58**: 537-545
[3]Bailey R.J.E. *et al.* (2006) *Canadian Journal of Fisheries Aquatic Science* **63**: 448-456
[4]Brooker A.J. *et al.* (2013) *Parasitology* **140**: 756-770

Poster #20

Determining the surface area of Atlantic salmon, *Salmo salar*

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The surface area of a host can influence the parasitic load of sea lice (*Lepeophtheirus salmonis*) on Atlantic salmon, *Salmo salar*. A wrap method combined with Image J software was used to determine the surface area of Atlantic salmon that had been used in a sentinel cage study. A mathematical model was then developed to estimate the surface area of individual fish based on weight using MatLab programming. The current mathematical formula fits fish surface areas more accurately than previously developed models. Earlier models are still cited for surface area estimation and could underestimate the surface areas of larger fish.

Poster #21

A drug resistance assay using copepodid-stage sea lice larvae

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Drug resistance in sea lice is a problem for the aquaculture industry but where treatments are unsuccessful care should be taken to eliminate other possible causes and to confirm resistance as opposed to possible user error. *In vitro* bioassays allow us to quantify drug sensitivity under standardised conditions, but there are practical problems with current approaches. Cage-side bioassays can be imprecise and inaccurate because it is difficult to obtain sufficient numbers of lice at the same stage of development. By comparison a more time-consuming and expensive test using an F_1 generation raised at a support lab can yield EC_{50} and LC_{50} values and confidence intervals that allow comparison over time and distance.

We are collaborating on an evaluation of a novel test for drug resistance using copepodids. These larvae could be obtained quickly by hatching egg strings collected at cage sites, or in larger numbers by hatching from egg strings obtained once female lice have been grown on fish in a laboratory environment. A copepodid bioassay method could have the advantage of accuracy over farm bioassays and of speed over F_1 testing. The approach will be extended to include use of multi-well plates and imaging technology. Once optimised this assay could combine good resolution with efficient use of resources.

Poster #22

Salmon lice infection on wild salmonids in marine protected areas: an evaluation of the Norwegian 'National Salmon Fjords'

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In Norway, 29 fjords have been protected in order to prevent the infection of important populations of wild salmonids with salmon lice of farm origin. We evaluated the effect of this protection on the lice infection pressure for wild salmonids based on lice counts performed on wild-caught sea trout and Arctic charr inside one third of these protected fjords (known as 'National Salmon Fjords'). Results indicate that these areas may provide a certain extent of protection against lice of farm origin, but their configuration will play a key role in their success. When the size and shape of a protected area are such that fish farms are kept at a minimum distance (calculated here as at least 30 km, but this distance might be strongly site-dependent), wild fish remain unaffected by the direct lice infection pressure imposed by fish farms. In contrast, the level of protection achieved in small protected fjords was limited, and we found a clear correlation between lice levels on wild salmonids in these areas and lice production in nearby salmon farms. To establish more precise management practices, both in National Salmon Fjords and other fjord systems along the Norwegian coast, the development and validation of accurate distribution and abundance models for the dispersion of planktonic lice larvae is needed; this could also be the basis for an area management system based on 'maximum sustainable lice loads' or 'lice quotas.'

Poster #23

Insights into the olfactory system of the ectoparasite *Caligus rogercresseyi*: Molecular characterization and gene transcription analysis of novel olfactory transduction pathway

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Although various elements of the olfactory system have been elucidated in insects, it remains practically unstudied in crustaceans. Among crustaceans, some species are classified as ectoparasites that impact the finfish aquaculture industry. Thus, there is an urgent need to identify and comprehend the signaling pathways used by these in host recognition. The present study reports novel transcripts involved in the olfactory system from *Caligus rogercresseyi* using RNA-seq and qPCR analyses. In addition, transcriptomic patterns during different stages of salmon lice development were evidenced. From a transcriptomic library generated by Illumina sequencing, contigs that annotated for a glutamate receptor, ionotropic kainite; an ionotropic receptor; a chemosensory protein; and other genes implicated in the olfactory system were identified and extracted. Full length mRNA was obtained for the ionotropic glutamate receptor 25, which had 3923 bp, and for the glutamate receptor, ionotropic kainate 2, which had 2737 bp. Furthermore, two other transcripts identified as glutamate receptor, ionotropic kainate 2-like were found. *In silico* analysis was performed for the transcription expression from different stages of development in *C. rogercresseyi,* and clusters were constructed. Gene transcription data were validated through qPCR assays in all the transcripts from the whole pathway. Several genes showed differentially regulated transcription levels among the different stages of salmon louse lifecycle, especially following the infective copepodid stage. This work contributes to the available knowledge on chemosensory systems in this ectoparasite, providing novel elements towards understanding the attachment process of the salmon louse, *C. rogercresseyi*.

Poster #24

Identification of candidate genes with response to delousing drugs by transcriptome mining in the salmon louse *Caligus rogercresseyi*

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Controlling infestations of copepodid ectoparasites in the salmon industry is increasingly problematic given higher instances of drug resistance or loss of sensitivity. Despite the importance of this issue, the molecular mechanisms and genes implicated in resistance/susceptibility are only scarcely understood. The objective of the present study was to identify and evaluate the transcription levels of candidate genes associated with delousing drug responses in the salmon louse *Caligus rogercresseyi*. From RNA-seq data obtained for adult male and female salmon lice, 62.48 M reads that assembled for 70,349 contigs were identified. BLASTX analysis against UniprotKB/Swiss-Prot and the ESTs available for crustaceans in the NCBI database identified 870 transcripts previously related to genes associated with delousing drug responses. Furthermore, 14 candidate genes were validated through qPCR and were evaluated with deltamethrin and azamethiphos bioassays. The results evidenced an overexpression of genes involved in ion transport in salmon lice treated with deltamethrin, while those treated with azamethiphos evidenced an overexpression of genes such as *cytochrome P450*, *carboxylesterase*, and acetylcholine receptors. The present study provides genomic relevant information to monitoring sensitivity to pyrethroids and organophosphates in a highly prevalent pathogenic species of the Chilean salmon industry.

Poster #25

RNA-Seq analysis evidences a multiple gene response in *Caligus rogercresseyi* exposed to the organophosphate Azamethiphos (Bayer)

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Azamethiphos is an organophosphate compound used for *Caligus rogercresseyi* control in the Chilean salmon industry. The molecular target of organophosphates has mainly been associated with the nervous system affecting acetylcholinesterase (AChE) activity. However, it has been demonstrated that in arthropods these chemicals could induce complex genomic responses, which can involve different molecular pathways not exclusively related to the nervous system. Our study presents transcriptomic analysis of *C. rogercresseyi* exposed to Azamethiphos and identifies novel candidate genes that are highly regulated as an effect of this delousing drug. Illumina sequencing was performed for adult male and female salmon lice exposed to 3ppb of Azamethiphos. We identified 16,302 transcripts highly regulated, which were annotated using BLASTX analysis against UniprotKB/Swiss-Prot and the EST-databases available for crustaceans from NCBI. RNA-seq analysis evidenced strong regulation in genes such as *cuticle protein*, *P-glycoprotein*, *Serine/threonin kinases*, *Myosin*, *NADH deshidrogenasa*, *ABC transporters* and *Glutathione S-transferase*. Furthermore, single nucleotide polymorphism (SNP) mining was carried out in genes with different transcription levels among treatments and sexes. The present study provides relevant genomic information from *C. rogercresseyi* in response to azamethiphos, allowing the identification of relevant genes associated with resistance/susceptibility to organophosphates.

Poster #26

Deltamethrin (AlphaMax) reveals modulation of genes related to oxidative stress in the ectoparasite *Caligus rogercresseyi*: implications on delousing drug effectiveness

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Deltamethrin is an insecticide authorized for use against the copepod ectoparasite *Caligus rogercresseyi* in cultivated salmonids (*1*). The degradation of this insecticide is principally catalyzed by P450 enzymes, carboxylesterases, and glutathione-S-transferase (*2*), which together increase the amount of free radicals that could promote the activation of an oxidative stress response (*3, 4*). The present study evaluated the transcriptomic expression of genes related to the antioxidant system in male and female *C. rogercresseyi* exposed to deltamethrin. Thirty-three transcripts were identified from an EST-database, including *Cr*GST, *Cr*GST-kappa 1, *Cr*SOD, C*r*CAT, *Cr*SE-GPx, *Cr*PHGP1, *Cr*PHGP2, *Cr*PRX6, *Cr*PRX2, *Cr*PRX4, and *Cr*FERR. Additionally, expression during five stages of development in *C. rogercresseyi* was evaluated *in silico* through RNA-seq analysis, and three clusters of genetic expression were revealed. The expression of *Cr*GST and *Cr*PRX2 was principally associated with the early stages of larval development, while *Cr*SE-GPx and *Cr*PHGP1 evidenced greater expression in adults. Moreover, the transcript expression for 11 genes of the antioxidant system was evaluated through qPCR in salmon lice exposed to three concentrations of deltamethrin. The results revealed that a 2 ppb concentration of the insecticide significantly increased the transcriptional activity of *Cr*SOD, *Cr*CAT, *Cr*PRX6, *Cr*PHGP1, and *Cr*PHGP2 in *C. rogercresseyi*. These results suggest that deltamethrin modulates the expression of genes belonging to the antioxidant system in salmon lice, promoting the generation of free radicals for the degradation of the insecticide. Additionally, sex differences reported are also discussed to evaluate their impact on developed protocols for evaluating the sensitivity and effectiveness of chemical control.

References

[1]SAG. (2014) Registro de productos farmaceuticos de uso veterinario, *Gobierno de Chile, Ministerios de Agricultura, Servicio agricola y Ganadero.*

[2]Sogorb, M. A., and Vilanova, E. (2002) Enzymes involved in the detoxification of organophosphorus, carbamate and pyrethroid insecticides through hydrolysis, *Toxicology Letters 128*.

[3]Ray, D. E., and Fry, J. R. (2006) A reassessment of the neurotoxicity of pyrethroid insecticides, *Pharmacology & Therapeutics 111*, 174-193.

[4]Dinu, D., Marinescu, D., Munteanu, M., Staicu, A., Costache, M., and Dinischiotu, A. (2010) Modulatory Effects of Deltamethrin on Antioxidant Defense Mechanisms and Lipid Peroxidation in *Carassius auratus* gibelio Liver and Intestine, *Arch Environ Contam Toxicol 58*, 757-764.

Poster #27

Increasing transcriptome response of SERPINs during the ontogenetic stages in the salmon louse *Caligus rogercresseyi*

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Serine proteinase inhibitors, or SERPINs, target serine proteases and are important regulators of intra- and extracellular proteolysis (*1*). For parasite survival, parasite-derived protease inhibitors have been suggested to play essential roles in evading the host's immune system and protecting against exogenous host proteases (*2*). The aim of this work was to identify SERPINs via high throughput transcriptome sequencing and elucidate their potential functions during the lifecycle of the salmon louse *C. rogercresseyi*. Eleven putative, partial SERPIN sequences in the *C. rogercresseyi* transcriptome were identified and denoted *Cr-serpin1* to *11*. Comparative analysis of the deduced SERPINs-like amino acid sequences revealed a highly conserved reactive center loop region. Interestingly, P1 residues suggest putative functions involved with the trypsin/subtilisin, elastase, or subtilisin inhibitors, which evidenced increasing gene expression profiles from the copepodid to adult stage in *C. rogercresseyi*. Concerning this, *Cr-serpin10* was mainly expressed in the copepodid stage, while *Cr-serpin3, 4*, *5*, and *11* were mostly expressed in chalimus and adults stages. These results suggest that SERPINs could be involved in evading the immune response of the host fish. The identification of these SERPINs furthers the understanding of the immune system in this important ectoparasite species

References

[1]Kanost, M. R. (1999) Serine proteinase inhibitors in arthropod immunity, *Developmental & Comparative Immunology 23*, 291-301.

[2]Armstrong, P. B. (2006) Proteases and protease inhibitors: a balance of activities in host–pathogen interaction, *Immunobiology 211*, 263-281.

Poster #28

Effect of salmon lice on sea trout in years of high and low infestation pressure

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Sea trout are especially susceptible to salmon lice infestations as they feed during their marine migrations solely in coastal waters, close to fish farms. Salmon lice infestations can have profound negative effects upon wild populations of sea trout as a result of reduced fish growth, stress effects and homeostatic perturbations, and increased risk of mortality due to behavioral changes. Despite the potential of decimating or even eradicating existing wild populations of sea trout, there are few studies on the population effects of salmon louse. In 2013, we caught and tagged around 1000 wild sea trout post smolts with PIT-tags in Etnefjord, western Norway. Approximately 50 percent of the fish were treated with a parasiticide before release. The live recaptures in sea were recorded and, in addition, an in-river PIT-tag detection system was installed in the main watercourse entering the fjord, river Etne, to record returning tagged trout. In 2014 we will repeat the design and tag and treat an equal amount of sea trout post smolts. The study years were chosen due to expected large difference in lice infection pressure as a result from synchronized fallowing in nearby fish farms. In 2013 sea lice levels on wild trout in the area was generally low, but is expected to be considerably higher in 2014 due to full production in all nearby fish farms. Recapture data and river detections from the two treatment groups will be compared both within and between years. This will add quantitative knowledge about possible population effects on wild sea trout caused by sea lice infestation.

Poster #29

In silico a analysis of lipid metabolic pathways during the ontogenetic development in the sea louse *Caligus rogercresseyi*

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The sea louse *Caligus rogercresseyi* life cycle includes vital moments for its parasitic success such as ecdysis or molt, hence we searched for genes directly engaged in lipid metabolic pathways involved in energy production and ecdysteroid synthesis. Genes were identified by high-throughput transcriptome sequencing analysis applied to different life stages of the ectoparasite. Among the 284 genes identified as belonging to the target pathways, 30 genes were selected to represent and describe the pathways, and these were annotated for proteins or enzymes involved in lipid digestion, absorption, and transport; fatty acid degradation; the synthesis and degradation of ketone bodies; and steroid and ecdysteroid synthesis. The putative lipid metabolism pathway was characterized based on differential transcription in the studied ontogenic stages and for feeding habits. Copepodids showed low expression of fatty acid digestion genes, reflected by a non-feeding behavior, and the upregulation of genes involved in steroid biosynthesis, consistent with a pathway for cholesterol synthesis during ecdysis. Chalimus showed an upregulation of genes from fatty acid digestion, absorption, and transport as well as fatty acid degradation and the synthesis of ketone bodies, suggesting a metabolic activity of the lipids absorbed from diet. Adult females were also linked to a pattern of high lipid metabolism for energy supply and mobilization in relation to reproduction and vitellogenesis. Different energetic needs between adult female and male were reflected in different lipid metabolism patterns. This study reports the probable lipid metabolic pathways involved in the energy production and ecdysteroid synthesis from the sea louse *C. rogercresseyi*.

Poster #30

miRNA Biogenesis pathway reveals transcriptional modulation in salmon louse (*Caligus rogercresseyi*) exposed to antiparasitic drugs

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Major challenges in the control of salmon lice has led to the increase of genomic information available for different species such as *Caligus rogercresseyi*, offering the possibility to develop more comprehensive studies to explore novel mitigation strategies against this ectoparasite. In this context, we identified and characterized a group of genes that mediates the post-transcriptional gene silencing conducted by microRNAs (miRNA) in *C. rogercresseyi*. Furthermore, RNA-seq analysis from male and female adults exposed to the antiparasitic deltamethrin (Alphamax) and azamethiphos (Bayer) were assessed. *In silico* analysis evidenced 24 putative genes involved in the miRNA biogenesis pathway such as transport, maturation and action of miRNAs. Among them, 243 putative single nucleotide polymorpshims (SNPs) were identified; of which 15 SNPs were non-synonymous mutations. With respect to RNA-Seq analysis, the CCR4-Not complex subunit 3 (CNOT3) was upregulated at earlier developmental stages (nauplius I-II and copepodid), and also after the exposure to azamethiphos, but not to deltamethrin. In contrast, the subunit 7 (CNOT7) was upregulated in response to deltamethrin, but not to azamethiphos. In addition, different Argonaut transcripts were associated with chalimus and adult stages, revealing specific expression patterns in response to antiparasitic drugs. Our results reveal novel insights into the post-transcriptional gene silencing in *C. rogercresseyi* mediated by miRNAs, and evidence a putative modulation of transcripts triggered by deltamethrin and azamethiphos. Future studies will evaluate the molecular underpinnings between the modulation of miRNA pathways and the resistance/susceptibility to delousing drugs.

Poster #31

Monitoring and characterization of resistance towards chemotherapeutants in the fish parasite *Caligus rogercresseyi* in Chile

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The sea lice *Caligus rogercresseyi* represents a major threat to the Chilean salmonid aquaculture industry. Losses in anti-sea lice treatment efficacy have been reported when using deltamethrin and emamectin benzoate, and reduced sensitivity, determined by means of bioassays, has been suggested (Bravo et al. 2008, Helgesen et al. 2014). In 2013, azamethiphos was introduced in Chile to improve the anti-louse treatment efficacy. Therefore, monitoring the resistance towards these chemicals should be a priority. Little is known about the molecular mechanisms behind resistance in *C.rogercresseyi*. The current project will focus on identification of resistance and - if detected - characterization of the resistance mechanisms in *C. rogercresseyi* against four antiparasitic drugs (deltamethrin, azamethiphos, emamectin benzoate and hydrogen peroxide). The methods and expertise developed through the work with *L. salmonis* will be applied. Results will provide important tools for resistance monitoring and thus provide a basis for more informed application of control measures.

The first step for the development of the project was to perform bioassays for monitoring *C. rogercresseyi* resistance and simultaneously select sensitive and resistant lice against the four aforementioned drugs. Adult female and male lice were collected from several salmonid farms in southern Chile (Region X) during March 2014. Methodology for bioassays followed Helgesen *et al.* (2014) protocol with some modifications (24-h bioassay using five or two doses plus controls). Reduced sensitivity was found towards deltamethrin and emamectin benzoate, but not towards hydrogen peroxide. For azamethiphos, some of the results pointed towards an early stage of resistance development. This hint needs further verification.

References

S. Bravo, S. Sevatdal, T.E. Horsberg 2008. Sensitivity assessment of *Caligus rogercresseyi* to emamectin benzoate in Chile. Aquaculture 282: 7-12

K.O. Helgesen, S. Bravo, S. Sevatdal, J. Mendoza, T.E. Horsberg 2014. Deltamethrin resistance in the sea louse *Caligus rogercresseyi* (Boxhall and Bravo) in Chile: bioassay results and usage data for antiparasitic agents with reference to Norwegian conditions. J. Fish Dis. doi: 10.1111/jfd.12223

Poster #32

Description of a novel *Lepeophtheirus salmonis* subsp*. salmonis* protein: A putative candidate for vaccination?

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The salmon louse *Lepeophtheirus salmonis* is a major problem in farmed Atlantic salmon, causing important economic losses and episodically serious injuries on fish. The parasite has developed resistance towards chemicals used for controlling it, making it difficult to reduce infection burdens. Alternative solutions, such as vaccination are therefore highly desired. This strategy however, requires that suitable parasite antigens can be identified and used in the vaccines. In this study, we present a novel *L. salmonis* subsp. *salmonis* protein that may be used in vaccination. We used a phage display approach for selecting relevant louse proteins. The technique involves the preparation of a parasite protein library displayed on the surface of T7 bacteriophages. The recombinant T7 phages in the phage display library are then tested for potential binding to fish blood proteins (an important part of the diet of the louse) such as complement factors and immunoglobulins.

We describe here one of the proteins selected by using this technique. Sequence analysis of this candidate has been completed (molecular and protein characterization). qPCR results indicated that the protein is similarly transcribed along the different developmental stages, including adult females and males. *In situ* hybridisation analysis showed that the protein is expressed in cells of the subcuticular tissue, intestinal wall and in some glands of the louse legs. Immunohistochemistry technique revealed the presence of the protein in testis, subcuticular tissue and intestine, mainly in cell nuclei. Functional analyses of the candidate have been performed via *in vivo* RNAi assay (post-transcriptional gene silencing). The RNAi results indicated that reproduction was significantly reduced when both preadult females and males were RNAi treated. Immunogenicity analysis of the candidate is in progress in mice and salmon.

Poster #33

The effect of chemotherapeutic drugs used to control sea lice on the fecundity rate of *Caligus rogercresseyi* (Boxhall & Bravo)

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The effect of four chemical treatments used in the control of sea lice on the fecundity rate of *Caligus rogercresseyi* was assessed. Samples of gravid females were collected from farmed rainbow trout in a site located in southern Chile (Region X). Gravid females were exposed to two different doses of deltamethrin, emamectin benzoate, azamethiphos and hydrogen peroxide, in addition of one control group. The medicines were dissolved in filtered seawater. In each flask (1 L) 12 gravid females were distributed with supplementary aeration; at temperature of 12ºC and a photoperiod of 12 h light and 12 h darkness. After 24 h of exposure, the mortality of females was evaluated and the eggs-strings were collected. The condition of the egg-strings was evaluated and classified as immature (unpigmented), mature (pigmented) and under hatching, before transferred to Petri dishes for hatching. Eggs-strings were incubated in fresh filtered seawater at 12ºC and assessed daily for a period of seven days. Abortion (nauplius-I developed into the egg but not hatched) was observed in 57.1% and 55.6% of the total egg-strings for each deltamethrin concentration (0.2 and 1 ppb respectively); 43% and 42% for emamectin benzoate (100 and 500 ppb); 43.8% and 50% for azamethiphos (0.4 and 2 ppb), and 50 and 35.7% for hydrogen peroxide (21 and 42 ppm). The remaining eggs-strings did not show development on maturation. Hatched live nauplii-I were recorded only at 0.4 ppb of azamethiphos, but they showed slower movements when compared with nauplii-I not exposed to chemicals. Nauplii-I emerged from the eggs during the 24 h antiparasitic drug exposition were found dead at the time of collection of the eggs-string for incubation in fresh sea water. All the egg-strings in the control group hatched and nauplii developed to copepodids. Similar results were reported for *Lepeophtheirus salmonis* by Toovey and Lyndon (2000), and Aaen et al. (2012). Results will be correlated with the sensitivity level recorded on females towards the four chemicals under study.

References

Toovey J.P.G, Lyndon A.R. 2000. Effects of hydrogen peroxide, dichlorvos and cypermethrin on subsequent fecundity of sea lice, *Lepeophtheirus salmonis*, under fish farm conditions Bulletin of the European Association of Fish Pathologists 20:224-228.

Aaen S.M., Bugge J., French M. 2012. Hatching of egg-strings exposed to hydrogen peroxide (Interox Paramove 50, Solvay). 9th Sea Lice Conference. Bergen, Norway.

Poster #34

Reference transcriptome and expression profiles for *Caligus rogercressiyi*

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[1]*University of Victoria, Victoria, BC, Canada*

The salmon louse, *Caligus rogercresseyi*, is an economically important ectoparasite of farmed and wild salmon throughout the southern hemisphere. Losses due to *C. rogercresseyi* have greatly affected production costs in the aquaculture industry. In order to better understand the life-cycle of this parasite, we characterized its transcriptome and to some extent its genome.

In this study, we report on a NGS analysis of *C. rogercresseyi*. We assembled a broad reference library consisting of mixed tissue, chalimus, copepodid, eggs, male and female samples. 45,476 putative transcripts were identified and subsequently, 9,422 full-length genes were characterized. Expression patterns were analyzed and library-specific differentially expressed genes of interest are reported. Complete expression profiles along with the raw data, have been submitted to NCBI's GEO database for public access. Additionally, we assembled and characterized NGS libraries from *Caligus clemensi*, Pacific *Lepeophtheirus salmonis*, and Atlantic *Lepeophtheirus salmonis*, and provide comparisons of functional enrichment. For the genome sequence, extensive polymorphism has resulted in low contig N50 sizes and we will report on efforts to overcome this problem.

Poster #35

Evaluation of the performance of pyrethroids on different life stages of *Caligus rogercresseyi* in southern Chile

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Control of sea lice in Chile is largely based on antiparasitic treatments, synthetic pyrethroids being the most used drugs. In recent years, farmers in Chile have reported decreased performance of pyrethroid-based treatments. The aim of this study was to assess the performance of two deltamethrin-based (Alphamax® and a generic product) and one cypermethrin-based (Betamax®) product on the different life stages of *Caligus rogercresseyi*, while controlling potential confounders. We found that both deltamethrin products and the cypermethrin product had a significant effect on the reduction of juvenile, mobile adult, and gravid female lice, compared with untreated pens; however, the effect on juvenile lice was less than on mobile stages. There was no evidence that pyrethroids performed better on a particular mobile life stage. When the three products were compared, no significant differences were observed in the numbers of juvenile, adult male, and non-gravid female lice after we controlled for potential confounders; however, cypermethrin exhibited a small, yet significantly greater effect on the gravid female group when compared with one of the deltamethrin-based products. We also confirmed that other factors besides the product choice, such as the pre-treatment sea lice abundance, water temperature and salinity, and time elapsed to the post-treatment sample, affect the post-treatment sea lice level as well; therefore, they should be taken into consideration when assessing the effect of immersion treatments.

Poster #36

Effectiveness of freshwater as a treatment in the control of *Caligus rogercresseyi* (Boxshall & Bravo)

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Sea lice were collected from two salmon farms located in Region X, Chile (41º44'LS; 73º09'LW) to assess the tolerance of *Caligus rogercresseyi* to freshwater treatment. The first study was carried out in December 2012 at site A, where salinity reached 26‰. The second study was carried out in April 2013 in Site B, where salinity reached 32‰. In the first study the tolerance of males and females exposed directly to freshwater (0‰) was assessed, and also the tolerance of *C. rogercresseyi* infesting rainbow trout (*Oncohrynchus kisutch*) exposed to bath treatment with freshwater for 30 minutes. In the second study the tolerance of males and females to a different gradient of salinity was assessed, and also the tolerance of copepodids to reduced salinity. In the first study 100% of males and females were immotile after 30 minutes of exposure. However, once they were returned to seawater the survival in males was 93%, 73% in gravid females and 100% in females without egg strings. In the second study a survival of 45% of females was recorded and 20% of males kept at 15‰ salinity for 24 hours. However, copepodids were not able to survive at a salinity of 25‰, which agrees with the information reported for *Lepeophtheirus salmonis* in the northern hemisphere (Tucker *et al*. 2000). The efficacy recorded for the detachment of *C. rogercresseyi* from rainbow trout treated with freshwater (0‰) was only 57%, with a higher tolerance of females in comparison with males. Lice collected from the site with 26 ‰ salinity, showed major tolerance to freshwater compared with lice collected from the 32 ‰ site, which corroborates the findings of Bravo *et al*. (2008).

References

Bravo S, Pozo V, Silva M. 2008. The tolerance of *Caligus rogercresseyi* to salinity reduced in southern Chile. Bulletin of the European Association of Fish Pathologists 28:197-204.

Tucker CS, C Sommerville, R Wootten. 2000. The effect of temperature and salinity on the settlement and survival of copepodids of *Lepeophtheirus salmonis* (Krøyer, 1837) on Atlantic salmon, *Salmo salar* L. *J. Fish Dis*. 23: 309-320.

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Poster #37

Drug susceptibility assessment of sea lice by time-to-response toxicity analysis

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[2]*Marine Environmental Research Laboratory, Machrihanish, Scotland, United Kingdom*

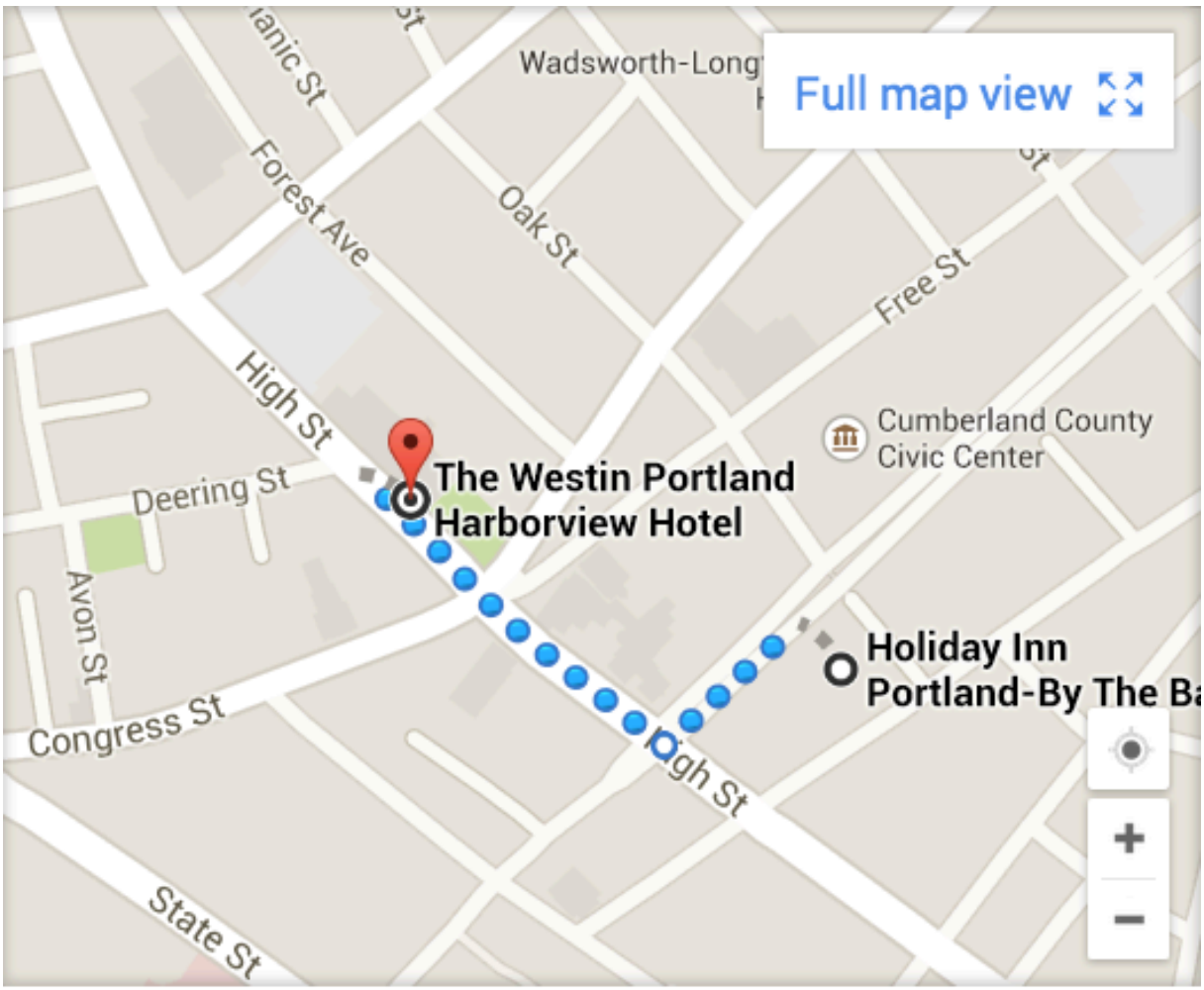
Sea louse control currently relies to a large extent on chemicals. However, the prolonged use of the same or similar acting compounds can favour the development of drug resistance. Successful prevention and management of drug resistance requires the deployment of well-established methodologies to assess the drug susceptibility status of target species populations. Current standardised sea louse bioassays provide such a methodology but require relatively high numbers of parasites. The present study explored the use of bioassays based on time-to-response toxicity analyses that can be carried out with limited number of test animals, using two laboratory strains of the salmon louse (*Lepeophtheirus salmonis*) differing in susceptibility to the drug emamectin benzoate (EMB). In time-to-response toxicity assays using adult male parasites, the median effective time (ET_{50}) of the immotility response to 800 mg L^{-1} of EMB was 6.9 h (95% confidence limits: 6.3-7.6 h) in the susceptible strain S and 23.5 h (95% confidence limits: 20.6-26.7 h) in the resistant strain R. By comparison, EMB EC_{50}s in the standard 24 h immobilisation bioassay were 73.9 µg L^{-1} (95% confidence intervals: 58.9 - 92.0 µg L^{-1}) and 778 µg L^{-1} (642 - 957 µg L^{-1}), respectively, in the S and R strains. In addition to a ~50% reduction in the number of test animals required, the novel assay offers the advantage of allowing to estimate the degree of drug susceptibility at the level of individual parasites, a feature that may be useful in studies aiming to identify molecular determinants of resistance.

Conference Events: Information

Conference Reception: *Sponsored by* Fish Vet Group
Where: Westin Portland Harborview
When: 5:30 pm to 9:30 pm Sunday August 31, 2014

Come join us and your fellow conference attendees for drinks and appetizers at the welcome reception at the Westin Portland Harborview Hotel. Enjoy folk music by local band “Old Grey Goose”. When not out on the water growing oysters and mussels this duo has played around the state and region entertaining many with their music.

The Westin Portland Harborview Hotel is a short 4 minute (0.2 mile) walk from the Holiday Inn by the Bay. To get to the Westin from the Holiday Inn take a left out of the main entrance and walk up Spring Street to the corner of High Street. Take a right onto High Street and walk 1.5 block up to the entrance to the Westin.



Conference Tours: Wednesday September 3, 2014

Odyssey Whale Watch

A Maine Whale Watching adventure awaits you aboard the Odyssey! Great fun and educational for the entire family. Join our Captain as he sets course on a journey to view those majestic mammals in their own environment. Enjoy the ocean air, narration and sights of Casco Bay as we cruise up to 20 miles out to known whale feeding grounds.

Odyssey Whale Watch has agreed to put on a special whale watching tour just for conference attendees. The tour will leave from their dock at 9am. They have agreed to give 10% discount to the normal tour price. Visit their website http://odysseywhalewatch.com/ or call (207) 775-0727 to make your reservation. There are a limited number of spaces.

Aquaculture Tour of Casco Bay

Maine has the largest marine aquaculture industry in the United States. Come join the Maine Aquaculture Association on a trip to one of the nearby mussel farms. The farmer will take you aroung the site and explain how we farm mussels in Maine. The executive director of the association will also be on hand to talk about the aquaculture industry in Maine. Don't miss this great opportunity to see a farm up close!

The tour will leave from the lobby of the Holiday Inn at 9am then board the Lucky Catch to cruise through Casco Bay to Bang's Island Mussel Farm. To reserve your spot email Rhonda at Futureseas@aol.com. Cost for the trip is $42 and it is limited to 14 people.

Traditional Maine Lobster Bake: *Sponsored by* Merck Animal Health
Where: Peaks Island
When: 5:00 pm to 9:00 pm Wednesday, September 3, 2014

This event will be held on the beautiful Peaks Island in the middle of Casco Bay. Transportation from the Casco Bay Ferry terminal to/from Peaks Island upon a private charter ferry, **which sails at 5pm is included (please be at the terminal for 4.30pm)**. The ferry terminal is a short 15-minute (0.8 mile) walk along the waterfront from both of the conference hotels, Holiday Inn By the Bay and the Westin Portland Harborview Hotel.

After getting off at Peaks Island it is a short walk (about 1/8 of a mile) to Greenwood Gardens, a former turn-of-the-century summer playhouse converted into a charming, rustic facility. The site provides seating inside the playhouse building, under the pavilion, and outdoors, overlooking the harbor and the Portland skyline. Play volleyball or horseshoes, wander the island's roads and paths, or just relax and watch the boats on the bay.

You'll enjoy a classic Down East feast of luscious lobster with all the trimmings: steamed clams, drawn butter, clam broth, corn on the cob, coleslaw, boiled potatoes, rolls, coffee, tea, punch, and fresh Maine blueberry cake. For those who prefer, steak, chicken, or vegetarian meals are also available. The steak and chicken feasts include everything except the clams. The vegetarian feast of pasta with sauce includes a fresh garden salad, corn on the cob, coleslaw, boiled potato and roll. Please indicate your meal choice at the time of registration. Everything is served in casual buffet style. Non-alcoholic beverages of coffee and lemonade will be served and a cash-bar will be available on site to purchase alcoholic beverages. **The private charter ferry leaves the island at 8.30pm returning to Portland Ferry Terminal for 9pm**, giving attendees the chance to sample the nightlife of Portland, Maine.

Please make sure you are at the Casco Bay Ferry terminal no later than 4:45 pm. The private ferry departs at 5pm.

Directions to the Ferry Terminal

The ferry terminal is a short 15 minute walk (0.8 mile / 1.3 km) from either the Holiday Inn or the Westin. The ferry terminal is located on Commercial Street. The simplest way to get there is to take a right out of the entrance of the Holiday Inn and walk down Spring Street, which turns into Middle Street to Franklin Street. Make a right onto Franklin Street and the ferry terminal will be right in front of you where Franklin deadends at Commercial (Fig 1).

Alternatively you can walk down Spring Street to Center Street and make a right onto Center. Center street both Pleasant Street and Commercial Street. Pleasant, which turns into Fore Street, runs parallel to Spring Street and Commercial taking you through the heart of Old Port section of Portland. You can take any right from Pleasant/Fore Street down to Commercial Street, which runs along the waterfront. The ferry terminal is a large brick building on the waterfront side of commercial.

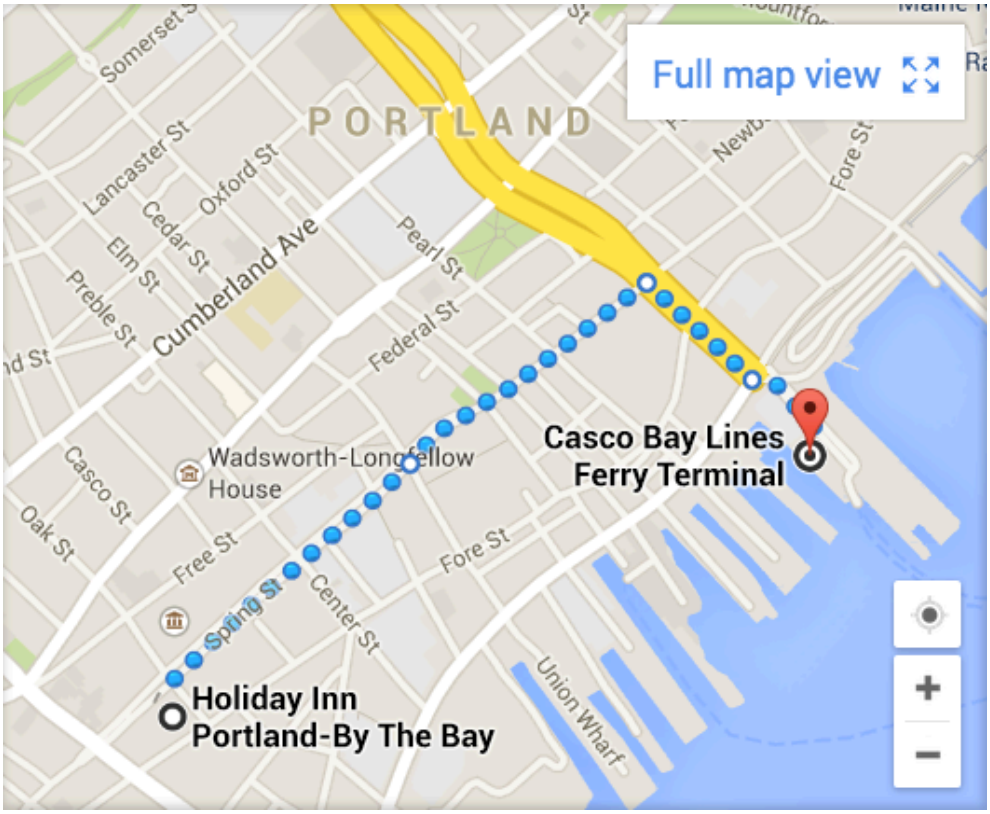


Figure 1. The simplest route to the Ferry Terminal.

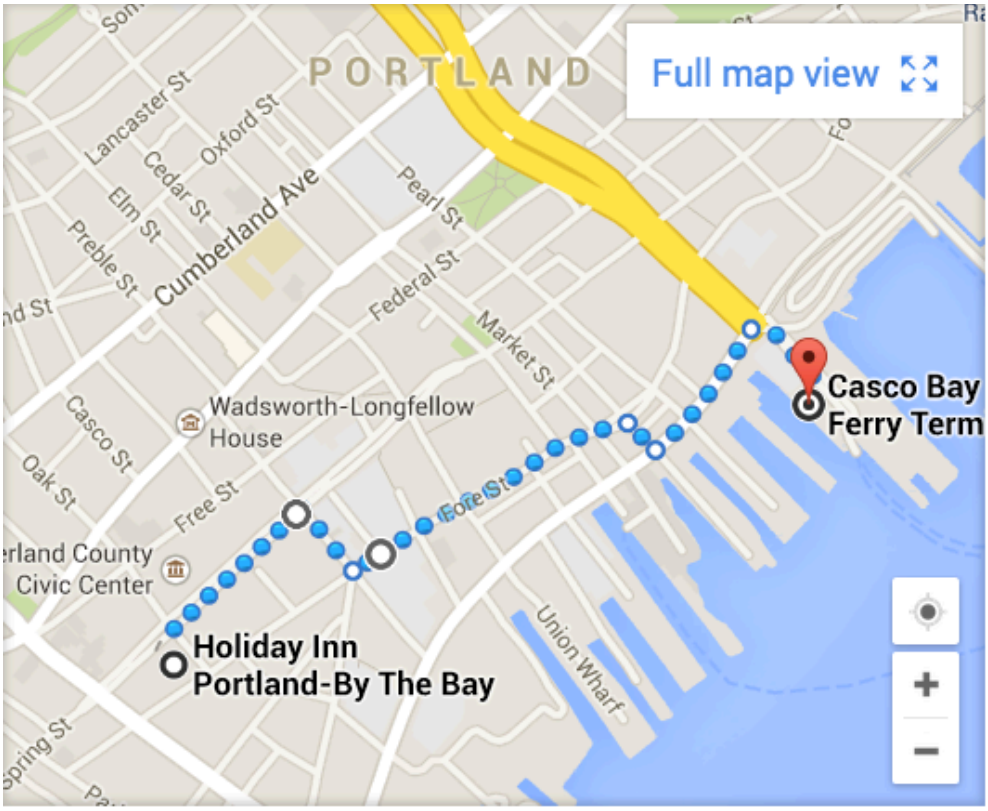


Figure 2. One possible alternative route. Down Spring St to Center St and then along Pleasant/Fore St and down to Commercial St.

Conference Banquet and Dance
Where: Holiday Inn by the Bay
When: 6:00 pm to 12:00 am Thursday, September 4, 2014

Come celebrate with old acquaintances renewed and news ones established throughout the conference at the 'Banquet and Dance' on the final evening of Sea Lice 2014. The banquet will be held at the Holiday Inn By-the-Bay and will feature a delightful meal. Please indicate your choice between a meat, fish or vegetarian option at the time of registering. After dinner join the DJ on the dance floor to show your best moves and let your hair down! Your banquet ticket not only includes the meal, music and dancing, but also two free drink tickets. Once you have used your free drink tickets a cash bar will be available throughout the dinner and dance.

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